THE 100 YEARS OF THE FUNGUS COLLECTION
MUCL
1894-1994

Fungal Taxonomy and Tropical Mycology:
Quo vadis ?

Taxonomy and Nomenclature of the Fungi

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1894-1994 MUCL Centenary

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LAST LECTURE, 5 December 1995

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Centenary Celebration of the Mycotheque de l'Université Catholique de Louvain, Belgium, 1894-1994

JOHN WEBSTER

University of Exeter, United Kingdom

MUCL is a fungal culture collection founded in Louvain in 1894 by Philibert Biourge. The centenary of the inauguration of this important collection is celebrated at a special symposium attended by some 150 guests from many countries at the Catholic University at Louvain-la-Neuve on Wenesday 29th June 1994. There are two sessions of presented papers, followed by a discussion, a poster presentation and an exhibition tracing the history of the collecton.

The morning sesssion is openend by addresses of welcome from Dr H. Naveau, Head of Applied Chemistry and Bioindustry Department of the Faculty of Agricultural Sciences and Dr P. Rouxhet, Prorector of UCL. Dr Rouxhet explains the philosophy behind the setting up of the University at Louvain-la-Neuve. It is an integrated community of academic staff and their families, students, residents, shops, private bisinesses and a science and industry park. Priority is given to scientific research and development, advanced technology, etc., with spin-offs likely to be benefit to the University. The Faculty of Agricultural Sciences, which receives state support, includes graduate school forming chemists and engineers able to apply their skills to the living world. The Prorector paid a large tribute to the dedication and hard work of the present director of the culture collection, Professor Grégoire Laurent Hennebert, and assured him that the collection would continue to receive University support. Professor Hennebert then traced the history of the culture collection over the past century since its foundation by Biourge, who had studied with Pasteur. A large number of distinguished scientists had been on the staff including Dierckx and Biourge who laid the foundation of the classification of *Penicillium*, Vandendries who worked on sexuality of Basidiomycetes, Wieldiers who discovered the "bios", a growth factor for yeast, later found composed of the B vitamins, Cappuyns whose work with the strains of *Penicillium* species and *Aspergillus niger* from the Biourge's collection established the industrial process of citric acid p-fermentation, Simonart, former director of the collection and present at the celebration, who worked with Raistrick of Oxford on the chemistry of *Penicillium griseofulvum* and discovered the first antifungal antibiotic the griseofulvin. The rest of the morning session is also devoted to papers on the significance of fungus culture collections. Prof. D.L. Hawksworth (Director of the International Mycolgical Institute, IMI) speaks about "Fungal resource collections and biodiversity", Dr. D. van der Mei (Director of the Centraalbureau voor Schimmelcultures, CBS) "The fungus culture collections in Europe", and Dr J. De Brabandere (Federal Service for Scientific Affairs, Belgium) "The Belgian Co-ordinated Collections of Microorganisms".

The afternoon session is a workshop entitled "Fungal taxonomy and tropical mycology: Quo vadis?" After an introduction by Pr. Hennebert, Dr C.P. Kurtzman

(NRRL, USA) and Dr. M. Blackwell (President MSA, USA) express the benefits of phylogenetic analysis for fungal taxonomy, Dr. J. Rammeloo, (National Botanical Garden, Belgium) and Dr. M.-F. Roquebert (National Natural History Museum, France) show the both the historical foundation up to the development of new means of the morphological approach in fungal taxonomy, Dr. A.J. Masuka (Forest Research Centre, Zimbabwe), Dr. A. Peerally (University of Mauritius) and Dr. J. Mouchaca, in name of E.J. daSilva (MIRCEN, UNESCO, France) develop the strategies in the development of mycological research and of fungus collections in the Tropics. In the subsequent discussion much concern is expressed about the lack of funding for taxonomic research on fungi and in the training of future generations of fungal taxonomists not only in developing tropical countries but also in developed temperate areas.

The exhibition of the history of MUCL was very interesting, including the notesbooks of Biourge, some of the original apparatus and microscopes used in his first laboratory, portraits, photographs, manuscripts, paintings and drawings, and publications, and a most attractive display of colourful and stricking fungal cultures.

The celebration ended with a reception in the grounds of a ruined Cistersian monastery, followed by a banquet in the adjacent Hotel des Ruines. The guests were entertained during the banquet by singers and dancers who were refugees from Rwanda. The British Mycological Society President, Professor A. Walley, presented Professor Hennebert with a ceramic sculpture of *Polyporus squamosus* on behalf of the BMS Council, thanked him for his hospitality and for organizing such a splendid celebration and gave the MUCL good wishes and continuing success for the next century of their activities.

PLENARY SESSION

PART 1

MYCOTHEQUE DE L'UNIVERSITE CATHOLIQUE DE LOUVAIN (MUCL):
100 YEARS OF EXISTENCE AND ACTIVITY OF THE FUNGUS CULTURE COLLECTION

Grégoire L. HENNEBERT

Mycothèque of the Catholic University of Louvain, Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

The Mycotheque of the Catholic University of Louvain is a fungal culture collection of filamentous fungi and yeasts.

It was started by Professor Philibert Biourge, microbiologist at the Brewery High School of the University in 1892. From contaminated beers, malts and barleys he purified many strains of beer yeasts and isolated wild yeasts, filamentous fungi and bacteria that he maintained in the collection. The collection was publicly inaugurated on July 8th, 1894.

After a well-known preliminary study of *Penicillium* Link by his assistant F. Dierckx (1901), Biourge prepared a full monograph of the genus *Penicillium* (1920, 1923). Later Professor P. Simonart made biochemical investigations on the *Penicillium* species in the collection and discovered the first antimycotic antibiotic griseofulvin from *Penicillium griseofulvum* Dierckx (1937). After Biourge's death in 1942, Pr. Simonart named the collection "Mycothèque Philibert Biourge".

In 1968, Pr. Simonart brought the collection together with the fungal collection begun in 1956 by G.L. Hennebert, and which already included 11 000 strains and herbarium specimens. In 1968 Pr. J. Meyer's fungus culture collection of *Hyphomycetes* from Central Africa, and in 1984, the collection of beer yeasts initiated by Biourge and maintained by the UCL Laboratory of Brewery Sciences were also incorporated.

In 1968 the Collection was officially recognized as the "Mycothèque de l'Université Catholique de Louvain" (MUCL) by the University, in 1969 by the International Association of Plant taxonomy (IAPT), in 1972 by the World Federation of Culture Collections (WFCC), and registered at the World Data Center (WDC). Since 1983, the MUCL Collection has been financially supported by the Science Policy Office of Belgium. It is a member of the Belgian Coordinated Collections of Microorganisms (BCCM), together with the collection of medical fungi at the Institute of Hygiene and Epidemiology (IHEM) in Brussels, the one of bacteria from the Laboratorium for Microbiology, University of Ghent (LMG) and the one of plasmids at the Laboratory for Molecular Biology, University of Ghent (LMB). From 1983 MUCL also has been a member of the European Culture Collections' Organization (ECCO) and from 1985, a partner in the Microbial Information Network Europe (MINE).

The MUCL culture collection

The MUCL Collection currently holds 24000 strains from all groups of filamentous and yeast fungi, including *Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes, Blastomycetes Hyphomycetes* and *Coelomycetes*. Most of the living

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strains originated from fungal specimens collected throughout the world and kept in the MUCL mycological herbarium which includes 38000 specimens of which 24000 are as living strains: 22000 filamentous fungi and 2000 yeast fungi, representing 3500 species of fungi and yeasts. The second edition of the catalogue was issued in 1992 with about 8000 strains[1].

The MUCL strains are maintained in at least two different ways, depending on the taxonomic group and on their effective sporulation. All strains are maintained on agar slants under mineral (parafin) oil. Strains producing spores in culture are lyophilized. Delicate and important strains are also maintained frozen at -80° C.

MUCL is a research-based culture collection. Its staff is currently carrying on research in both systematic and applied mycology.

Systematic mycology:
- Collection and isolation of fungi in unexplored sites and substrates throughout the world, particularly in extreme environments and in Africa.
- Morphotaxonomy, chemotaxonomy and nomenclature of yeasts and filamentous fungi, particularly Blastomycetes, Hyphomycetes and Aphyllophorales.
- Morphogenesis and morphology of sexual and asexual reproduction.
- Floristics and ecology of fungi in their natural habitats (thermal waters, burned sites, sea sand, rocks, dung, resins, leathers, dried fish, tar, all kinds of industrial products and residues).
- Physiological, biochemical and biomolecular characterization of fungi and yeasts for taxonomy and biotechnology, implemented into a new expert system of identification.
- Biochemical and biomolecular taxonomy of yeasts and filamentous fungi: protein and enzymic profiles, coenzyme Q, RFLP, RAPD mapping, rRNA and rDNA sequencing, phylogenetic analysis.
- Development of a fungal data base on Macintosh (software 4D) following the MINE formats.

Applied Mycology:
- Food contamination. Post-harvest deterioration in storage of cereals and legumes. Flour and dairy contamination. Food fermentation and starter production (cheese, kefirs, sourdoughs, tempeh, fermented cassava, beers and wines).
- Cultivation of edible mushrooms. Production of spawn. Improvement of production and nutritional value. Domestication of edible non-cultivated tropical species.
- Industrial, agricultural and domestic waste biodegradation, agricultural composting and industrial recycling.
- Characterization of wood-decaying fungi. Deterioration of material by fungi (wood, paints, plastics, textiles, etc). Fungal resistance testing of materials. Bioassays of fungicides. Diagnoses in industries and buildings. - Association of termites and fungi.
- Monitoring of product contamination in agricultural and industrial environments. Improvement of production and storage conditions.
- Characterization and production of mycorrhizae for reforestation in the tropics. Production of starters for inoculation.
- Screening of fungi in search of particular properties for biosynthesis.

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[1] The Mycotheque preserves presently over 51.000 herbarium specimens and 35.000 living strains of fungi

- Improvement of preservation techniques of living strains of fungi (freeze-drying and cryopreservation).

References

Biourge Ph. 1920. Les moisissures du groupe *Penicillium* Link, étude monographique (Conference, Louvain, 3 April 1916) *Bull. Ass. Anc. El. Ec. Sup. Brass. Univ. Louv.*, **20**(3): 99-127.

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Dierckx F. 1901. Essai de revision du genre *Penicillium* Link. Note préliminaire. *Annales Soc. Scient. Brux.,* **25**: 83-89.

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Hennebert G.L. 1985. Dierckx' Contribution to the Genus *Penicillium.* In *Advances in Penicillium and Aspergillus Systematics*, Samson, R.A. and J.I. Pitt, eds., 9-21., 4 fig. Plenum Press N.Y. and London.

MUCL 1894-1994 step by step

1891-1892 Philibert Biourge, born on April 8, 1864, is Dr in Botany UCL, and is awarded a scientifi research stay at the Pasteur Institute in Paris, under the direction of Louis Pasteur and of Drs Roux and Duclaux on the pure culture of microorganisms.

1892 On return from Paris Ph. Biourge is nominated assistent of Prof. Jean-Baptiste Carnoy , Professor of Botany and Microbiology, UCL. (photo) . Carnoy was author of "Recherches morphologiqueset physiologiques sur les champignons" (Bull. Soc. Roy. Bot. Belg.9-2:157-311, 1870).

1892 Ph. Biourge makes a second study stay in Denmark by E. Christian Hansen wo was applying the monospore technique of Pasteur for the purification of *Saccharomyces cereviviae* from the wild yeasts.

1892-1894 Ph. Biourge initiates a Culture Collection of Microorganisms, yeasts, filamentous fungi and bacteria from beers and cereals, at the Laboratory of Zymotechny of the Brewery High School, Catholic University of Louvain, in Louvain.

1894 Public inauguration of the Biourge's Culture Collection with exhibition of living cultures of yeasts, filamentous fungi and bacteria, at the Brewers Congress, Antwerp, 8 July 1894 (*Journal du Petit Brasseur*, 2 (19) suppl., p.7-11, July 8, 1894, photos).
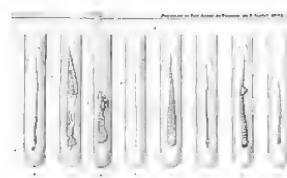
1895 Biourge studies the microbial alterations of beers and of the products of alcoholic fermentation at the Laboratoire de zymologie de l'Ecole Supérieure de Brasserie de l'UCL (photos) (Ph. Biourge, "Les maladies microbiennes de la bière" *Bull. Assoc. Anc. El. Ec. Sup. Brass. Univ. Louv.*, 1(1): 1-4. "Recherches sur la fermentation alcoolique" *La Cellule,* 11: 93-109). Applying single cell culture method from Pasteur and Hansen, Ph. Biourge is purifying the Belgian beer yeasts for breweries.

1896 Ph. Biourge is nominated Professor UCL.

1897 Ph. Biourge develops the culture collection of moulds, *Penicillium* and others, from malts. Oscar Semal study fungal production of ammonium (*La Cellule,* 13(2): 284-312, 1897). René Vandendries presents a PhD thesis on the "Matières colorantes azotées chez les Champignons".Vandendries will be one pioneer in the sexual mechanism in the Basdiomycetes from 1923 to 1937,

1898-1901 François Dierckx (1863-1937) makes a taxonomic study of *Penicillium* species of the Biourge's collection.

1899 Death of Prof. Jean-Baptiste Carnoy (1836-1899).
Prof. Ph. Biourge becomes head of the Laboratory of Microbiology, Faculty of Sciences, UCL.

1900 F. Dierckx (photo) presents a PhD thesis on 26 new species of *Penicillium* with drawings and publishes "Essai de revision du genre *Penicillium* Link. Note préliminaire", *Anns. Soc. scient. Brux.,* 25(1): 83-89, 1901. He showed his type cultures to P.A. Saccardo, on his way to Indonésia. but lost all his belongings including the cultures in that country. Only one duplicate type culture left in Louvain, *Penicillium griseo-roseum* Dierckx, could be recovered by Biourge.

1901 Ernest Wieldiers, Md Dr, another Biourge's collaborator, discovers the "Bios", an extract of yeast that contains the required vitamins B, K and others for the growth of yeast. Wieldiers E. "Nouvelle substance indispensable au développement de la levure" *La Cellule,* 18(2): 311-333, 1901.

1902 The "Question of Bios" is a matter of dispute among scientists. A. Amand, collaborator of Biourge, answers the dispute (*La Cellule,* 20(2): 223-251, 1902).

1903-1914 Ph. Biourge develops his collection of *Penicillium* strains. He collected again the species described by Dierckx on basis of Dierckx drawings left in Louvain. He also gathers other *Penicillium* types from Zaleski, Whemer, Thom, Bainier and Franz Krâl Collections (Krâl started in 1884 a private collection of several hundreds of bacteria, yeasts and filamentous fungi at the Institute of Hygiene of the Faculty of Medecine at the Prague University. It was one of the earliest collection in the world. After Krâl's death in 1911, the collection went to Vienna from where Ersnst Pribam got it in 1915 and published a first catalogue of it in1919).

1910-1914 Alphonse Cappuyns (1887-1936) starts biochemical researches on bacteria and fungi.

1914-1918   World war: Ph. Biourge retires in his landhome where he works on a *Penicillium* monograph. A. Cappuyns retires in a studio at Bruxelles where he selects a citric acid producing *Penicillium* strain from Biourge's collection.

1919   First industrial production of citric acid from a *Penicillium* strain by A. Cappuyns in "s.a. Les Produits Organiques de Tirlemont", Belgium, after the failure of such a production by the Fabrique de Produits Chilmiques de Thann et Mulhouse, France. (Mazé P. & Perrier A. 1904 *Anns. Inst. Pasteur*, 18:553-575)

1920   Ph. Biourge publishes a summary of his forthcoming monograph "Les moisissures du groupe *Penicillium* Link, étude monographique", *Bull. Assoc. Anc. El. Ec. Sup. Brass. Univ. Louv.*, 20(3): 99-127, 1920.

1923   Ph. Biourge publishes his *Penicillium* monograph with 126 species "Les moisissures du groupe *Penicillium* Link, étude monographique", *La Cellule,* 33: 3-331, 1923.
Ph. Biourge receives the *Grand Prix de l'Exposition International du Centenaire de Pasteur* à Strasbourg (photo)
        A collaborator, Victor Estienne presents a PhD thesis on *Aspergillus atro-ruber* (*Harzia acremonioides*).

1925   Ph. Biourge is also devoting much time to the study of the Dutch Elm disease: "La maladie des ormes. *J. Soc. cent. Agric. Belg.,* 73: 22-44, 1925.

1926   A. Cappuyns started a new selection of an acid-resistant citric acid producing strain to substitute it to the too instable *Penicillium* strain in use: "Sur la formation d'acide sulfurique libre dans les cultures de certains microorganismes.", *Anns. Soc. scient. Bruxelle*, 45: 177-183, 1926).

1927   Biourge is working on the manuscript of a monogrraph of the *Aspergillus* as "*Brevis Conspectus generis Aspergillus Link*" and a preliminary paper entitled "Monographie culturale macroscopique des Aspergillus" with 13 colour plates describing the colours of 208 strains on two media, of which three original plates are preserved at MUCL.

1929   New and successful start of "La Citrique Belge" in Tirlemont by A. Cappuyns (photo), from a selected strain of *Aspergillus* group *niger* from the Biourge's Collection.
        Paul Simonart (1907-1998), collaborator of Biourge, starts biochemical studies of the lactic fermentation.

1931-1934 P. Simonart is awarded with Prof. H. Raistrick in London. They publish the discovery of the gentisic acid in the biochemistry of *Penicillium griseofulvum* Dierckx: Raistrick H & Simonart P. Studies in the biochemistry of microorganisms 29. 2:5-Dihydroxybenzoic acid (gentisic acid) a new product of the metabolism of glucose by *Penicillium griseofulvum* Dierckx. *Biochem. J.* 27(3): 628-633, 1933);

1932   Raoul Mosseray makes physiological and morphological studies on the *Aspergillus* group *niger* for PhD: "Influence du zinc sur les *Aspergillus* de la série *niger* et sur quelques autres." *La Cellule* 41: 11-128

1933   Biourge provides in his paper "Sur les champignons dits moisissures. A quoi bon leur étude et comment la faire.", *Rev. Quest. scient.*, 52(103): 53-78, a justification of a fundamental and taxonomic study of the fungi, in view of their large physiological and biochemical potentialities.

1934   P. Simonart defends his PhD thesis on "The biochemistry of *Penicillium griseofulvum* Dierckx" at the University of London.
    From the *Aspergillus* strains collected by Ph. Bourge and collected by himself in Africa, Raloul Mosseray publishes the monographic study of "Les *Aspergillu*s de la Section *Niger* Thom et Churh." *La Cellule*, 43: 201-286. also *Ann. Soc. scient. Brux.* B, 54: 72-85. He also studies the "Races naturelles et variations de culture chez divers *Aspergillus*." *Ann. Soc. scient. Brux.* B, 54: 161-189.
    René Vandendries develops his study of the sexuality in Basidiomycetes
    Paul Henrard develops a PhD study on the sexuality of *Aspergillus nidulans* and *Eurotium* species, "Polarité, hérédité et variation chez diverses souches d'Aspergillus." *La Cellule*, 43(3): 350-324.

1935   P. Henrard studies further the sexuality of ascomycetes in "Observation sur le comportement de souches monascosporées de *Xylaria polymorpha* Pers. (Grev.)." *Broteria* 14(31): 79-83.
    P. Simonart, in collaboration with A.E. Oxford and H. Raistrick publishes the discovery of the "Fulvic acid, a new crystalline yellow pigment, a metabolic product of *Penicillium griseofulvum* Dierckx, *P. flexuosum* Dale and *P. brefeldianum* Dodge.and other species." *Biochem. J.* 29(5) 1102-1115.

1936   Ph. Biourge publishes his observations on *Ophiostoma ulmi* "Le cycle du champignon de la maladie de l'orme." *Ann. Soc. scient. Brux.* 56: 130-193.
    P. Simonart (photo) is nominated Professor UCL, and awarded Advanced Fellow of the Belgian American Educational Foundation. With Dr. Ch. Thom, in Washington, he is confronting the Biourge's *Penicillium* Collection to that of Thom.

1938   Ph. Biourge is Emeritus Professor. Prof. P. Simonart is responsible of the Laboratory of Microbiology and takes the Fungus Culture Collection in charge, but the Biourge's yeast collection is kept under care at the Brewery School.

1939   Ph. Biourge, G. Van Cutsem & E. Bredo put forwards *Ophistoma ulmi* as the cause of a humain infection, "Une mycose nouvelle: la graphiomycose." *Rev. Belg. Scienc. Médic.*, 11(5): 217-236.
    P. Simonart, in collaboration with A.E. Oxford and H. Raistrick publishes the discovery of the "Griseofulvin, $C_{17}H_{17}0_6Cl$, a metabolic product of *Penicillium griseofulvum* Dieckx." *Biochem. J.* 33(2): 240-248, the first antimycotic antibiotic.
    The Fungus Culture Collection moves, together with the Laboratory of Microbiology, from Carnoy Institute of Botany, Faculty of Sciences, to the new Institute of Agronomy. Prof. Simonart denominates the Collection "*Mycothèque Philibert Biourge*".

1940-1944   Second world war.

1942   Prof. Ph. Biourge passed away on April 19, 1942**.**

1945   The Mycothèque Ph. Biourge within the Laboratory of Microbiology, moves from the old Institute in Louvain city to the new Institut Agronomique in the Parc van Arenberg in Heverlee near Louvain.

1949   Raymond Lambert (1923-1992), UCL assistant, then workleader in Prof. Simonart's Microbiology laboratory, takes care of the Mycothèque Philibert Biourge up to 1960.

1950-1960 Joseph Meyer (1924-), Dr in Botany, studies soil microfungi, Hyphomycetes and others, of Central Congo, in Yangambi, Congo, establishing a herbarium and a culture collection. (photo)

1956   Grégoire L. Hennebert (1929-), FNRS research fellow at UCL, initiates a personal Fungus Culture Collection in the Plant Pathology Laboratory, UCL Faculty of Agronomy, and starts a research on *Botrytis* and the Sclerotiniaceae with Prof. V. Estienne as promotor.

1960   Joseph Meyer, collaborator in Prof. Simonart's Laboratory, takes care of the Mycothèque Philibert Biourge, until 1969, as well as of his collection of African Hyphomycetes.
    G.L. Hennebert presents the thesis "Recherches morphologiques sur le genre *Botrytis* Persoon", for obtention of the PhD graduation at UCL.

1960-1962 G.L. Hennebert is awarded by the Canadian National Research Council a two-year postdoctoral fellowship at the Mycology Unit of the Plant Research Institute, Ottawa, with Dr Stanley J. Hughes (photo).

1962 G.L. Hennebert Visiting Research Mycologist, Plant Pathology & Mycology Dept., Cornel Univerity, Ithaca, NY.

1962-1965 G.L. Hennebert is awarded FNRS qualified researcher and appointed UCL teaching assistant in Plant pathology, developing the Fungus Culture Collection, at the Faculty of Agriculture, Heverlee-Leuven. (photo)

1963-1975 MUCL develops a close collaboration with the Central Bureau voor Schimmelcultures CBS in Baarn.

1964 Visite of Dr.C.J. Alexopoulos from to the Collection (24.08.1964).

1965 Visit of Pr. Dr. C.V. Subramanian from Madras to the Collection (23.06.1965).
G.L. Hennebert and J. Meyer are nominated UCL Professors.

1966 Visit of Pr. DR. R.P. Korf from Cornell University, Ithaca, NY. to the Collection (13.06.1966)

1967 G.L. Hennebert, visiting lecturer, University Lovanium, Kinshasa, Congo.

1968 The G.L.H. Collection is composed of 12,000 herbarium specimens and 7,000 living strains (Anonymous 1968. Centre d'Etudes: le Laboratoire de Mycologie Systématique et Appliquée. Nouvelles Brèves, REUL, Rel. ext. Univ. Louvain, 18 Juin: 3-4)
Prof. Simonart transfers the Mycothèque Philibert Biourge to the G.L. Hennebert Fungus culture Collection. The Mycoth.èque Biourbe consists of 683 *Penicillium* strains and 122 A*spergillus* strains, a number of them having been returned to Pr. Simonart by Thom (USA) as earlier received from Biourge.
The UCL authorities agrees upon the new denomination of the integrated Collection of Fungi as the "*Mycothèque de l'Université Catholique de Louvain (MUCL)*", which becomes internationally recognized by the International Association for Plant Taxonomy (*IAPT Regnum vegetabile 1969*).

1969 Pr. J. Meyer contributed his Collection of African soil fungi to the Mycothèque.
Visit of DR. J.A. von Arx, CBS, Baarn, The Netherlands (19.04.1969)

1969-1986 G.L. Hennebert is effective member of the Special Committee for the Nomenclature of Fungi and Lichens of the IAPT-N.

1970 G.L. Hennebert is visiting Professor, Laval Univerity, Québec, and invited Professor Tchechoslovakian Academy of Sciences, Praha.

1970-1994 MUCL is member of the International Biodeterioration Research Group (IBRG) and of the Internationa Association for Wood Preservation (IRGWP)

1971 G.L. Hennebert is nominated UCL Professor.
Second Visit of Pr. Dr. C.V. Subramanian, India (28.09.1971).

1972 MUCL becomes affiliate of the World Federation of Culture Collections (*WFCC Directory ed. 1972*). A first catalogue of the included species represented by living strains is then provided.

1972-1973 Prof. Richard P. Korf is awarded Fulbright-Hays Senior Research Scholar and stay six months at MUCL and the UCL Faculty of Agriculture (photo). He delivered a special conference on "A new classification of the Operculate Discomycetes"

1973 Visit of Dr. Stanley J. Hughes at MUCL (photo) for discussion on the classification of Fungi Imperfecti and collaborative work on *Sporoschismopsis* (3-17.05.1973).

1974 Creation and first issues of MYCOTAXON, an international journal for the taxonomy of fungi and lichens, by Dr R.P. Korf and G.L. Hennebert.

1975 Move of the MUCL Fungus Culture Collection to new laboratories of the Faculty of Agricultural Sciences on the new UCL campus in Louvain-la-Neuve, 30km south-east of Brussels.

1977 Publication of "Anamorph, teleomorph and holomorph, terms for forms of fungi, their names and types", defined by Hennebert and Weresub, *Mycotaxon* 6: 207-211.

1981-1990 Creation of the Laboratory of Microbiology at the University of Burundi, Faculty of Agronomical Sciences, Bujumbura, Burundi and establishment of the LMUB Culture Collection.

1981 G.L. Hennebert, Invited Professor at the Potchefstroom Univerity, South Africa

1982-1990 G.L. Hennebet**,** Visiting Professor in Microbiology, University of Burundi, Bujumbura.

1983 MUCL becomes a member of the European Culture Collection Organization (ECCO) and take part to the second annual ECCO meeting in Paris.
MUCL is a contractual partner, together with LMG and IHEM, of the *Belgian Coordinated Collections of Microorganisms* (BCCM) newly created by the Belgian Science Policy Office (SPPS) and supported by the Belgian State.
MUCL within BCCM, IMI and CBS with support of the SPPS, at Bangkok, are deciding the initiation of the project of the *Microbial Information Network Europe* (MINE) aiming the harmonization and computerization on line of the strain data available in European collections.

1985 The project MINE is approved by the CEC with MUCL, LMG and ICP in BCCM, CBS, CMI, DSM, IP, LCP as first partners. Among them MUCL, LMG and CBS elaborate the standard format for the database and published catalogue (photo) of the fungi and bacteria.

1986 G.L. Hennebert, Visiting Professor, Institut Agronomique et Vétérinaire d'Agadir, Maroc.

1987 Visit of Dr. M. van Uden (IGC, Portugal), Dr. S. Udagawa (NHL, Japan), DR. R.P. Korf (CUP, USA), Dr. L. Blaine (ATCC, USA); Dr. R. Haruenkit (TISTR, Thailand) to MUCL in Louvain-la-Neuve.

1989 First edition of the "MUCL List of Cultures Fungi-Yeasts 1989" of almost 5000 strains (photo).
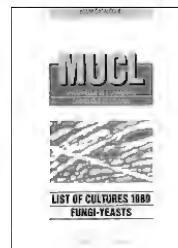
1990 MUCL is elected responsible of the MINE Committee for the harmonization of the computerized strain data on Fungi and Yeasts, under the BRIDGE EEC Programme.

Publication of "La Mérule, Science Technique et Droit" par G.L. Hennebert, Ph. Boulenger et Fr. Balon, Ed. Ciaco, 198 p. over the dry rot fungus problem.

1992 Second edition of the "MUCL Catalogue Fungi-Yeasts 1992" containing almost 8000 strains.(photo)
MUCL, and the other BCCM Collections, are elected as International Depository Authority (IDA) for the deposit of fungus and yeast strains for patent purposes under the Budapest Treaty by Belgian state decision..

1994 July 8. The Mycothèque de l'Université Catholique de Louvain is since 100 Years a public collection.
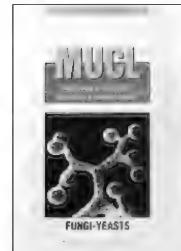
FUNGAL GENETIC RESOURCE COLLECTIONS AND BIODIVERSITY

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Abstract: Institutions or activities must reconsider and reinterpret their objectives in the language of the day; failure may endanger their survival. The single act of relabelling "Culture Culture Collections" as "Microbial Genetic Resource Collections" immediately makes a link with agendas of the 156 government signatories to the *Convention on Biological Diversity*. In addition, collections must be poised to answer fundamental questions: Who needs the collections and why? How effective is the present system? How many strains of a species are needed ? In what ways should collections collaborate to maximize effectiveness? And how can national and regional collections contribute to the sustainable use of the Earth's resources? In order to realize the potential of the collections, a fuller integration in which national, regional, and international facilities have complementary roles must be sought.

Introduction

In order to prosper, an institution or activity must repeatedly reconsider and reinterpret its objectives in the language of the day. And also in accordance with the agendas of its users and funding sources. However much a service is seen as invaluable and essential to those intimately involved in it, failure to be seen to relate to topical issues risks increasing marginalization, and a falling away of the very constituency essential to its survival. Microbial collections have no special exemption, and heed should be taken of the experience of some of our major museums and botanical herbaria.

Founded in 1894, the Mycothèque de l'Université Catholique de Louvain, whose centenary we are celebrating, is the oldest collection of fungal cultures still operating. If the Mycothèque had not been able to adapt to changing policy requirements, we would not be here today.

In the mid-1990s, demands for justification and accountability have become universal. In this climate, it is imperative that those involved in culture collection development and management are poised to present their case in a manner which is in harmony with the issues of today. Here, I pose questions that need to be addressed at levels from the individual collection to the national, regional, and international.

What should culture collections be called?

The label "culture collection" is a barrier to communication. It has hindered the forging of links with the rise of interest and funding devoted to the *ex-situ* conservation of plants and animals during the last two decades. Interdisciplinary meetings and reports on "genetic resources" have only exceptionally considered microorganisms, and it is proving a slow process to change the perception that fungi and microorganisms are something apart from "genetic resources".

Article 2 of the *Convention on Biological Diversity* defines genetic resources as "*genetic material of actual or potential value*"; "genetic material" being "*any material of plant, animal and microbial or other origin containing functional units of heredity*" (UNEP 1992). Article 9 (b) directs the contracting parties to the Convention to "*Establish and maintain facilities for ex-situ conservation of and research on plants, animals and micro-organisms, preferably in the country of origin of genetic resources*". A dictionary definition of a resource is "a source or possibility

of help"; that immediately conveys a positive message in contrast to "culture" which makes no value statement.

The phrase "culture collection" does not appear in the Convention. The single act of relabelling "culture collections" as "microbial genetic resource collections" makes an instant link with the agendas of the 156 governments that are signatories to the Convention; and also to other programmes starting to be developed as the ripples from Rio extend ever wider (e.g. Kapoor-Vijay 1992).

Who needs the collections and why?

Fungal genetic resource collections have a diverse customer base. However, while they are crucial at different points in time in many areas, a single customer will often use their services only rarely. The key needs are:
- to provide named material for industry and both pure and applied research in a timely and cost effective manner.
- to safeguard genetic resources as an insurance policy through holding stocks in long-term storage for future needs.
- for comparative purposes to make identifications and maintain type cultures[*] that fix the application of names.
- to maintain patent and industrial strains of microorganisms.
- to use in education and training.

Common myths must also be dispelled. There is a frequent misconception that fungi and other microorganisms are everywhere and just wait to be isolated. Were that true, desired fungi could be isolated from nature on demand. While that thesis may hold for opportunistic moulds associated with humans and their products, and perhaps some elements of the soil mycobiota (Gams 1992), such statements have to be kept in perspective. Quotations such as "everything is everywhere and the environment selects" (Baas-Becking 1934) can be profoundly damaging if not placed in context. In reality, around 70 % of the fungi are obligately associated with particular host plants or animals (including insects) as pathogens, commensals, mutualists, or specialized saprobes. Their distributions will thus tend to be closely correlated with those of their hosts.

In the case of fungi which are not visible to the unaided eye or with a hand lens, and which are isolated from nature by dilution-plate or similar methods (Gams 1992), the probability of refinding a species the spores of which occur at low frequencies is so remote as to be impractical.

Further, many fungi which can be seen by eye, forming macroscopic fruit-bodies or otherwise visible colonies, have only once or exceptionally been successfully brought into culture. In some cases this may not be technically difficult provided that isolations are conducted while the material is in a fresh condition - for example, isolated close to the point of collection before the specimen dries out. An example of what can be achieved by developing particular isolation strategies is a recent project involving lichen-forming fungi. Although around one fifth of all fungi have this biology, lichen-forming fungi are scarcely present in microbial collections. Yet 427

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[*] Although living cultures of fungi are not acceptable as nomenclatural types under the International Code of Botanical Nomenclature (ICBN), the latest edition of the Code (Greuter *et al.* 1994) makes clear by an example that cultures "permanently preserved in a metabolically inactive state", such as by lyophilization or storage in liquid nitrogen **are** acceptable; the Code recommends that cultures resurrected from such permanently preserved cultures are referred to as ex-holotype, ex-isotype, etc.

(42 %) of 1021 lichen-forming and lichen-inhabiting species attempted were successfully isolated, including representatives of many genera, families, and even orders never previously obtained in culture (Crittenden *et al.* in prep.).

Fungal genetic resource collections can maintain such rarely or not otherwise cultured strains so that they are available for both research and evaluation for potential applications. Pragmatically, the collections are the **only** source of pure living isolates of many fungi.

How effective is the present system?

One measure of the effectiveness of the present system is the number of species of fungi available in the world's service collections. In 1990 the collective holdings were estimated at 11 500 fungi, about 17 % of the 69 000 then described (Hawksworth 1991)[*]. If the conservative working estimate of 1.5 million fungi on Earth is accepted, something for which there is growing evidence (Hawksworth 1993), that means only about 0.8 % of those actually existing are currently in a service collection.

Fungi, as catalogued in the *Index of Fungi*, are currently described at the rate of about 1 700 species per year. Precise data are not available, but only about 30 % of these are known in culture. As the number of known species increases, on current trends the proportion represented in the service collections can be forecast to **decrease** rather than to increase. This situation could be at least partly rectified by mycologists endeavouring to prepare cultures immediately after collection, as strongly advocated by Huhtinen (1994), although major targeted isolation programmes (*see above*) may be necessary to make a significant impact on the current situation.

How many strains of a species are needed?

At 351 263, the number of individual strains of fungi available in the world's service collections at first seems impressive; on average that is 17 strains under each name indexed (Sugawara *et al.* 1993). However, this statistic does not automatically imply an enormous duplication in the genetic resource held. The seed banks of major cereals count their accessions in 100 000s, for example, with 333 413 accessions for *Oryza sativa* in 1991 (Groombridge 1992) in order to preserve the range of the genetic resources within a single species. Fungi also exhibit substantial variation within a species. The extent of infraspecific variation in a particular fungus is often unclear, due to the lack of investigation, but well-studied examples show that this can be expected to be considerable.

The mycelia from morphologically defined species are often unable to fuse together, forming "vegetative incompatability groups" in both sexually and asexually reproducing species (Brasier 1987). For example, Ploetz (1990) found 11 incompatability groups in 96 strains of *Fusarium oxysporum* sp. f. *cubense*. Stable aneuploids and polyploids may be a key factor in some cases, but chromosomes

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[*] The most recent edition of the *World Directory of Collections of Cultures of Microorganisms* (Sugawara *et al.* 1994) lists 19 392 names of fungi implying that a much higher proportion of species is held. Further, of the names of fungi listed, 9 539 are indicated as held in only one. However, these figures are inflated as they do not allow for synonyms, separately named anamorphs, alternative taxonomies, nor different spelling variants used in the collections submitting data.

receive scant attention from mycologists; indeed, aneuploidy was recognized in *Penicillium* only recently (Bridge *et al.* 1986).

The extent of variation at the molecular level can also be exemplified by *F. oxysporum,* where seven mtDNA RFLP patterns may occur within a single special form (Jacobsen & Gordon 1991). In the particularly well-studied *Neurospora crassa*, genetic stock collections maintain some 3000 different genetic variants of this single species (Fungal Genetics Stock Center 1988).

Against the background of this genetic variation, it is sobering to note that a study of the holdings of the various living fungal collections demonstrates that a considerable number of fungi are represented only by single isolates. An indication of this is provided by data compiled in the *World Directory of Collections of Microorganisms* (Sugawara *et al.* 1993) where 49% of the taxa are listed as represented in only a single collection. In the case of the International Mycological Institute (IMI) collection, 2541 (56%) of the 4541 species maintained are represented by only one isolate.

There is at present no general answer to the question "How many strains of a species are needed?" in fungi, but we can be confident that the figure is considerable. Were a modest 10 strains to be maintained for all 72 000 known fungi, that 720 000 would require a doubling of the collective holdings of **all** the world's service collections.

In what ways should collections collaborate to maximize effectiveness?

The *World Directory of Collections of Cultures of Microorganisms* lists 246 collections of fungi (including yeasts) world-wide (Sugawara *et al.* 1993). While this work does not include industrial and other research collections, this number approximates to those actively involved in the preservation and supply of fungus cultures today. These collections vary enormously in size, from a few hundred to more than 45 000 strains. Some are general in scope, but many specialize in fungi relevant to particular applied areas, for example plant pathology, medical mycology, genetic stocks, edible fungi, or brewing.

The collections are not evenly distributed geographically. Only 39 of the 142 countries wholly or partly located in the tropics have collections registered with the *World Directory*; while 107 collections are involved, 52 (49 %) are in one of two countries, Australia and India (Hawksworth & Ritchie 1993).

It has to be accepted that: (a) no single collection is ever likely to achieve the level of funding necessary to serve as a genetic resource collection for all groups of fungi; and (b) many countries are likely to remain without national collections for the foreseeable future unless appropriate funds can be released. If an adequate service to the world is to be provided, one which is able to fulfil all the criteria identified above, and further satisfy national needs, collaborative mechanisms must be sought.

The need for collaboration was foreseen in the *Convention*, the signatory governments agreeing under Article 9 (e), to *"Cooperate in providing financial and other support for ex-situ conservation"*. This call was not a new one; the UN Conference on the Human Environment, meeting in Stockholm in 1972, had already recommended action "*in respect of micro-organism germ plasms,* [to] *co-operatively establish and properly fund a few large regional collections*" (United Nations 1972). Co-operative action on the level required has not taken place, and nor will it now

unless the collections themselves develop a workable strategy in discussion with the donor community and national governments.

An indication of what could be achieved is provided by the International Plant Genetic Resources Institute (IPGRI; formerly the International Board for Plant Genetic Resources, IBPGR). IPGRI, founded in 1974, established a network of centres specializing in storing the seeds of particular crops in order to further the collection, documentation and use of germplasm for crop species (Williams 1988).

In the case of microbial collections, organizations and networks exist which can be used to ascertain what strains are held, including the European Culture Collection Organization (ECCO), the World Federation for Culture Collections (WFCC), Microbial Strain Data Network (MSDN), Microbial Information Network Europe (MINE), and various national organizations. The Biodiversity Information Network 21 (BIN 21; Canhos *et al*. 1992) also promises to ease the exchange of data on holdings. However, these bodies do not have the resources or mandate to control or coordinate their member collections. The UNESCO/UNEP-sponsored Microbiological Resource Centres (MiRCENs) scheme, establishing centres, especially in less developed countries (Da Silva 1991), is very much on the lines required but has been undersourced and will need to adapt to the changed situation with regard to property rights.

How can national and regional collections contribute to the sustainable use of the earth's resources?

Article 10 of the *Convention on Biological Diversity* places emphasis on "*the sustainable use of components of biological diversity*" and not only on its conservation. In addition to the general roles of genetic resource collections of fungi, they can contribute to sustainability at the national and regional level by:
- safeguarding the resource, especially from disappearing habitats.
- making the resource available for screening and use on the terms of the country of origin.
- utilizing the resource for biocontrol, waste degradation, and food production.
- maintaining mycorrhizal mutualists important for forest re-establishment.
- providing a source of reference strains to speed the diagnosis of plant pathogens.

Making the fungal resource available in a controlled way for screening purposes which will bring maximum income into a country is of especial significance. The issues are complex (Reid *et al*. 1993), and national legislations have yet to evolve. However, payments on a per strain basis linked to royalty agreements are possible. When the question of how to use a particular hitherto uncut forest in a sustainable way for the benefit of the local population is posed, isolating and selling on fungi is an option not to be discounted. In cases where an individual country cannot provide a national facility, agreements on a regional basis should be sought.

Conclusion

Microbial resource collections are **the** way of making fungal biodiversity available for exploitation in support of sustainable development. They are the catalyst required to turn potential into reality.

However, fulfilment of that goal will require both collaboration and the development of mechanisms to enhance the complementarity between collections. Collaborative networks at a variety of levels are emerging as the logical approach to the provision of biosystematic services in speciose groups (Janzen 1993, Jones 1994). Genetic resource collections have demonstrated, through MINE and other initiatives, that they can successfully combine and exchange data. We should not be complacent, and recognize the need to move to a second level of co-operation beyond that of networking data; to a fuller integration in which national, regional, and international facilities have complementary roles. As such a proposal would be in accord with current concerns as to the conservation and sustainable use of biodiversity, we should not be afraid of making major funding proposals. "Big science" funds with no immediate benefits to humankind are secured because of a unanimous clamour from the enthusiastic scientists involved; we need to follow suit and convey our vision of the potential to the donor community.

Acknowledgement

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THE FUNGAL CULTURE COLLECTIONS IN EUROPE: CHANCES, CHANGES AND CHALLENGES

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The celebration of the centennial of the "Mycothèque" of the Catholic University of Louvain-la-Neuve is in itself reason enough to congratulate the University and the "Mycothécaire", Gregoire Hennebert. I could leave it at this.

This centennial jubilee, however, concerns an Institution within a University, and a collection of fungi. And these two aspects ask for a somewhat deeper reflection.

To-day's universities are characterized by rapid changes, triggered by financial problems and short-term policies more often than by long-term considerations in which sciences should be embedded. This holds for most disciplines, but particularly so for systematic biology with as its main component taxonomy and as its major instrument the collections. Most universities, as a consequence, have in the recent history been not very accommodating media for these two.

In the last two decades the number of taxonomic staff in universities all over Europe and world-wide has dwindled, collections in particular being considered as questionable assets.

One of the reasons is that collections are rather expensive to keep, while the revenues, mostly measured as numbers of publications in highly esteemed international journals, are only to be seen in the long term and are seldom directly retraceable to the collection and the university that pays for it.

More important is that the scientific scene for many years has been dominated in biology by the so-called "reductionistic approaches", with as main components molecular and cell-biology. The great break-through, originating from these disciplines, has grasped the imagination of the public domain, masterminding the direction of research in virtually all biological subjects. The holistic approach, in which the organisms themselves in relation to their surrounding environment, living and dead, are the issue, has lagged behind.

The fact is that these two mainstreams, reductionism and holism, are still not well-balanced, making biology a divided house. This is in contrast to physics and chemistry. The holistic approach, of which collections - whether they contain living or dead material - are to be seen as a major component, has to gain a lot of lost ground in order to compete successfully for national and international shares of the budget.

Well over 100 years ago the Swiss author Rudolph Töpfer created his "Monsieur Cryptogame", internationalized as "Herrn Steckelbein" in Germany and as "Mijnheer Prikkebeen" in the Netherlands. He personified the "Collectioner" as a man deeply satisfied by the pleasure of sampling in Nature, and deeply unhappy with womanhood, expressed by his pathological fear for his sister Ursula. A caricature he was.

But when discussing collections and taxonomy with those who decide upon my budget for next year, I still encounter the deep-felt suspicion that the only reason for our existence is in playing Monsieur Cryptogame, the fear for sister Ursula maybe replaced by the fear for bureaucrats, still mainly motivated by gathering as many

organisms as possible for the pleasure we get from them and not bothering about the sense of it all.

The taxonomist, at the time of birth of Monsieur Cryptogame, stood at the cradle of *the* unifying concept in biology, the theory of evolution. And he still stands in the centre of evolutionary biology, looking for the roots and the history of biodiversity as it presents itself to-day. And he has grasped the opportunities that are offered by new techniques, especially in molecular biology, to tackle the problems more adequately. In taxonomy, and directly in relation to it, in collections, a major effort is undertaken to bridge the gap between reductionism and holism, for the benefit of biology as a whole.

I hope that in the near future the taxonomy, within the context of systematic and evolutionary biology, of life on the planet earth will reach on the scale of issues worthwhile investigating the same level as the taxonomy of our stellar system, for which billions of dollars are attributed easily in the construction and salvation of Hubble-telescopes and the like.

The fact that Biodiversity has appeared on the agenda of the United Nations is a milestone, as is the fact that taxonomy nowadays is firmly linked to the mainstream in biology.

Let's not be over-optimistic and self-confident. There are some major hurdles ahead, to mention a few:

1. Taxonomy and collections lack a strong organizational structure, that can act as a sparring partner speaking with a voice that is clearly heard.
   In the Netherlands, within the framework of the graduate school of biodiversity, botanists and zoologists are always to be reminded that there are also microorganisms and collections, besides botanical gardens and zoos, where living organisms are kept in perfect shape. There seem to be three taxonomies, that of animals, that of plants and that of microorganisms.
2. The expectations of those who put biodiversity on the political agenda may differ greatly from insights, expectations, possibilities and capacities of taxonomists and collections.
3. Biodiversity has the danger of becoming a fashion. Fashions in science may lead to a proliferation of activities, based upon status and "getting a slice of the pie", rather than upon sound scientific strategies and ideas. The fact that taxonomy is at the basis of this fashionable term is often overlooked.

Nevertheless, there is a revival of taxonomy, and together with that, a keen interest in what collections are and what they can do. It is up to us to show that we are up to the challenges, national, European and world-wide.

A recent report of the Royal Netherlands Academy of Arts and Sciences, in which its scientific priorities are reconsidered, still does not mention taxonomy as one of the main issues.

For survival of the bacterial collections in the Netherlands money has to be found exclusively on the free market and in contract-research.

These are also facts. Structural support has to come from reshuffling of budgets invested in medicine, chemistry and physics, disciplines which have well-structured organizations and lobbies. Without a fight we will not succeed.

My first words in this part of my lecture were to praise the Universities of Leuven and Louvain-la-Neuve for fostering the "Mycothèque" through the first 100 years. They have shown wisdom in doing so, and it is my sincere wish that they continue to support the Mycothèque for the next 100 years, as the only

"mycothèque" among many just plain European fungal "collections". And we even do not ask them to ensure that the "mycothécaire" should be allowed to go on till he can celebrate his centennial. We know him well enough that - apart from any official position - he will continue as the taxonomist in heart and soul that he has always been.

The main theme given to me to talk about is.

Fungal collections in Europe.

My expertise on this subject should not be exaggerated. On first sight it seems to be quite appropriate for someone who has been the coordinator of the Microbial Information Network Europe (MINE) project, aiming at bringing together some 40 collections in 12 European countries in the endeavour to set up a common format for the data on bacteria and fungi and to bring these data together at a Central Data Node. I have to admit that my view on this matter is still a bit blurred, MINE not always being illuminating in this respect. Notwithstanding the rather limited financial support from EC, and an underestimation of the complexity of the project, it is clear that:

1. There is great willingness to cooperate between collections.
2. The framework for optimal cooperation still has to be established.

In order to improve on this second factor, the good framework for cooperation, a number of facts and conditions should be kept in mind.

1. Collection policy is mainly determined at the national level, not in Brussels. Any cooperative approach should be based on a "Europe of Nations" not on a "Federal Europe". In retrospect, without having participated during the whole preparatory phase of MINE, I am convinced that this aspect has been neglected in the onset of the MINE project. After all, we must realize that, however important Brussels has become on the playground of subsidizing institutions in Europe, a role recently illustrated by the acceptance of the 4th Framework in which some 12.3 billion ECU's are to be spent on research for the next four years, it still does not cover more than 5% of the total input from public means in the countries of the European Union. The major impact of Europe is in cooperation and in stimulation, the necessary infrastructure still being a matter of the national governments.

2. Without cooperation and presentation of initiatives that are considered worthwhile and challenging in the pursuit of biotechnology, a sustainable use of natural resources, and in the competition with Asian and American counterparts, major inputs from the European Union in taxonomy and collections are, at least, questionable. This support is needed to cope with expected technical and methodological changes in collections.

3. Without cooperation and coordination, the diversity in European collections, which can be considered a great asset, both in the variety of organisms kept and in the expertise they represent, will become a hampering factor, not to be used to the maximum in the world's biodiversity programs.

4. Any future cooperation must include Eastern Europe. The borders of the European Union are not to be considered limitative.

5. Collections are fiercely competing organizations, not only in assets and their scientific value, but also - and increasingly so - on the free market.

This summary of statements proves that any framework for cooperation should accommodate the deeply felt necessity for working together bearing in mind that there are a number of hampering factors in this respect. There is a certain reluctance to be encountered when discussing input from taxonomy and collections in biodiversity research with EU officials. This was the case with MINE, and it can be clearly seen in the cautious approach in the concerted action for microbial biodiversity. And the underlying factor is mistrust in the potential for collections and taxonomists to come forth with coherent and well-defined proposals. Maybe not rightfully so, but the fact has to be acknowledged.

An overview of the major actors, professional groups as well as organizations, in the fields of taxonomy, collections and biodiversity research is represented here in the form of a human figure, the caption 'Quo Vadis?' indicating that the direction it will take is not certain yet.

The trunk consists of the scientific community and it financing bodies. The arms represent the major international organizations, the United Nations and the Unions of the International Council of Scientific Unions.

The head, becoming rather large, represents the politicians, civil servants, managers and lawyers.

The legs, symbolizing the European Culture Collections Organization (ECCO) and MINE are in contrast rather thin.

The driving factors are, of course, to be found at the heart: the taxonomist and the curator. They are supplied, symbolized by blood vessels and lungs, by enthralling ideas from many other scientific disciplines such as molecular biology and biotechnology, which in turn acknowledge the mighty impulses they get from the heart, recently revived by the bypasses biodiversity and evolutionary biology.

The discussions between these groups are not always easy, as illustrated by some imaginary conversations on biodiversity.

The molecular biologist has just received a DNA sample for identification from a zoo in some faraway country. He compares it with some DNA obtained from his colleague, the taxonomist. After one day he exclaims that in the country concerned they have the atrocity to keep human beings in zoos, as the DNA sample matches rather perfectly the DNA from the taxonomist. The taxonomist, who by nature is a curious man, travels to the zoo, and returns quite disappointed: the sample was that of a chimpanzee. Every one of us in this case is an expert: quite clearly the chimpanzee and the human being are different species, one bothering about his origins, the other, as far as we know, not.

However, when we are talking about microorganisms, the claim of the molecular biologist that he is the one who can unequivocally discriminate between species by applying his DNA trick is often acknowledged without dispute.

The biotechnologist and the taxonomist discuss biodiversity in a quantitative way, the taxonomist stating that there are over six million species on earth. The biotechnologist is hardly impressed. His biodiversity concept is governed by the enzymes and he knows that in evolution, through all kingdoms, from archaebacteria to the animals, has resulted in about 5000 different enzymes, subdivided in 60 groups and 6 classes. His biodiversity concept is much simpler, interested as he is in regulatory systems that lead to the production of enzymes in higher quantities and that do the tricks he wants them to perform, better. And then again he is primarily interested in strains, not in species. The direct surrounding of the taxonomist thus is

one in which different paradigms are amalgamated in an enriching way. The least that should come of it is a reconsideration of species concepts.

The discussions with the organizations constituting the rest of the body are quite different, but not less important in character.

They have to do with selling ideas in such a way that the product, of which we only are at the onset aware of its value, becomes attractive to those who should pay for it.

This process takes place at an increasing number of levels and is to a certain degree masterminded by politicians, managers and lawyers. It starts at the level of universities and research councils and ends at conferences in Mexico and Nairobi. It is sometimes tempting to look at these levels with some disdain, but fact is that all of us are devoting more and more time to travel around the world and participate in the circuit of buying and selling science. Without clear research-strategies the danger that the outcome is mainly determined by the buyer, is evident. When looking at biodiversity, the four main topics are: the scale, where? what? and how?

The scale

It is the world, but also the own back garden.

The most far-fetched proposal, the All Taxon Biodiversity Investigation may raise severe doubts about feasibility and scientific value; it has the scale that can grasp the mind of policy-makers, which surely can boost the impact of taxonomy, together with collections as the main actors in the biodiversity field, a role that seemingly cannot be attained by smaller projects.

Where?

The focus on the tropics is evident. There are, however, many other habitats that are of great interest and about which much is still unknown.

They should not be overgrown by over-emphasizing, strongly supported by public concern about its destination, the Rain Forest. The soil, the tundra, the sea are habitats in the temperate zones that ask for special attention of taxonomists and of collections in Europe too.

What?

Besides taxonomic issues about inventory, isolation and identification, evaluation of species concepts, and nomenclature, the collections in particular are confronted with questions on how to preserve, in situ and ex situ, what to preserve, and where.

This is accompanied by international questions about ownership of microorganisms, resulting form the Biodiversity Convention.

How?

There is a limited number of taxonomists, and a rather large number of collections, different in character and quality. Without good cooperation between collections, resulting in well-defined forms of quality control and division of labour and fields of activity, I am afraid that collections will find it even more difficult to present themselves in the field of biodiversity research than the taxonomists in general.

It is important to indicate strong - and consequently also weak - points in the European collections, and this should be done by collections themselves.

I have already stated that the legs are in bad shape: we have to worry about their capacity to carry the whole body steadily. The message is clear: the legs have to be strengthened.

Rather then finding new formulas, I am of the opinion that in principle the existing organizational structures, ECCO for more conceptual thinking, MINE for more technical matters should be adequate, on condition that they are well-equipped for their supporting roles. And this asks for a rather fundamental discussion and reshaping of both organizations.

ECCO should evolve from a yearly meeting of curators into an organization that can speak up for collections and is not afraid to raise issues that may be of some concern to the collections themselves.

MINE should be transferred into a consortium.

Most important is to find, for both organizations, a constitution that is recognized by all collections as fair, with boards that are equipped with a clear mandate to work with.

There is nothing new, only the form has changed and things are now more complicated. CBS originated in 1903 as a result from a concerted action by the international community of scientists to start something that is to the benefit of that community.

I am sure that we also will celebrate our centennial as proof that international cooperation pays off in the long run.

And if things become boring or too complicated we have always our inspiration from the organism that governs our daily lives, the Fungus. In itself it is beautiful and far more complicated than the structures I have been talking of.

THE BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS
BCCM

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The 'Belgian coordinated Collections of Microorganisms, BCCM' form a consortium of four complementary research-based culture collections at the service of the international scientific and industrial community.

The Belgian Science Policy Office launched the BCCM-action in 1983 with the following mission.

1. The characterization, inventory and preservation of the pool of available isolates, reference strains and plasmids (over 41.000 items), to be expanded along the lines of the specializations of each collection:
- the LMG bacteria-collection (Director: Prof. Dr. K. Kersters, Curator: Dr. D. Janssens) holding over 13000 strains;
- the MUCL collection for fungi/yeasts of biodiversity and (agro)industrial interest (Director/Curator: Prof. Dr. Ir. G.L. Hennebert) holding over 22000 strains;
- the IHEM collection for fungi/yeasts of biomedical interest (Director/Curator: Dr. N. Nolard) holding over 5000 strains;
- the LMBP plasmid-collection (Director: Prof. Dr. Ir. W. Fiers, Curarator: Prof. Dr. E, Remaut) holding over 1000 plasmids for use in bacteria, filamentous fungi, yeasts and animal cells.

2 The distribution of the authenticated biological material and the corresponding information to interested scientific and industrial circles.

3 The acceptance of patent deposits valid within the framework of the international Budapest Treaty (N.B. The BCCM received in 1992 - and this as a consortium - status of International Depositary Authority, IDA);

4 The valorization of the available skills in the field of both fundamental and applied (micro)biology by providing professional and confidential *ad hoc* services and contract-research to third parties (e.g. molecular characterization and typing of industrial and medical strains, safe deposits of propriety strains, training, screening for primary and secondary metabolites etc.).

All BCCM collections are active members of the "World Federation for Culture Collections, WFCC"' and the "European Culture Collections Organization, ECCO". Moreover, the BCCM are founding-members of the "Microbial Information Network Europe, MINE"'. As such, they host the "Data Integrating Node" for bacteria at Ghent and chair the "Responsible Committees" for bacteria and fungi/yeasts

The organization of the BCCM-consortium constitutes a rather unique model that distinguishes itself from foreign examples by the stable funding from one common financier that is also in charge of the central coordination and support of the main policy issues (client approach, informatics, quality management etc.).

PART 2

FUNGAL TAXONOMY AND TROPICAL MYCOLOGY: *QUO VADIS* ?
An Introduction

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Modern taxonomy, including mycotaxonomy, relies in an increasing degree on new molecular approaches.

As such, one can observe that the traditional morphotaxonomic approach is more and more complemented - or sometimes even substituted - by chemotaxonomic methods or genome-based analyses. Questioning the value and mutual complementarities of all available tools - both classical and modern - is essential to be able to handle the systematic classification problem in an integrated and effective way.
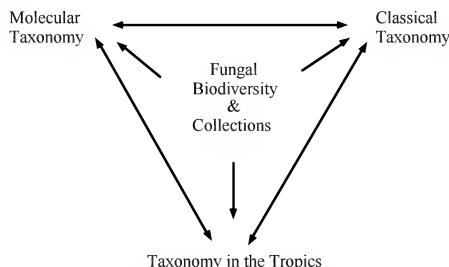
On the other hand, the greater part of the fungal biodiversity has still to be discovered and described especially in the tropical regions. As these are mainly situated in developing countries, one has to discuss - in the spirit of the Rio de Janeiro earth summit - key topics such as training, technology transfer and the set-up of local *'ex situ'* collections.

During such an exercise, special attention has to be given to taxonomic and preservation methods that are not only effective but also widely applicable, user-friendly and robust.

MOLECULAR TAXONOMY OF THE YEASTS: PRESENT AND FUTURE

CLETUS P. KURTZMAN

Introduction

The systematics of yeasts, as with other groups of fungi, is predominantly based on a hierarchy constructed from morphological differences among the taxa. The present system of classification relies heavily on types of budding or the occurrence of fission, presence or absence of hyphae, morphology of ascospores, and morphology of basidia and the manner in which basidiospores are produced. As a consequence, these characters often define orders, families, genera, and even species. Whether the characters reflect the evolutionary history of the yeasts is unknown, but if systematics is to be based on phylogeny, i.e., a natural classification, the genetic basis of currently used characters must be understood.

Application of molecular comparisons to questions in yeast classification offers an unprecedented opportunity to reevaluate current taxonomic schemes from the perspective of quantitative genetic differences. This review examines the impact of molecular comparisons, notably ribosomal RNA/ribosomal DNA (rRNA/rDNA) sequence divergence, on the current phenotypically defined classification of the yeasts.

Placement of yeasts among the fungi

The yeasts represent a unique group of fungi characterized by vegetative growth that is predominantly unicellular, and by the formation of sexual states which are not enclosed in fruiting bodies. *Saccharomyces cerevisiae* has been recognized as an ascomycete for well over a century, but it was not until much later that some yeasts were thought to be basidiomycetes (Kluyver and van Niel 1927). This supposition was confirmed by Banno (1967) with the description of the heterobasidiomycetous species *Rhodosporidium toruloides*. With this finding came the realization that the yeasts are phylogenetically quite divergent. In the following discussion, relationships among ascomycetous and basidiomycetous taxa are examined from the perspective of phylogeny determined from small and large (18S, 26S) subunit rRNA/rDNA sequence divergence. Methodologies have been discussed by Bruns *et al.* (1991), Kaltenboeck *et al.* (1992), Kurtzman (1992), O'Donnell (1992), and White *et al.* (1990).

Ascomycetous yeasts

The phylogeny of the ascosporogenous yeasts has been vigorously debated since the time of Guilliermond (1912) and before. Some have viewed the yeasts as primitive fungi while others perceived them to be reduced forms of more evolved taxa. Cain (1972) has been a proponent of this latter idea, arguing that hat (galeate)-spored genera such as *Pichia* and *Cephaloascus* are likely to be reduced forms of the

perithecial euascomycete genus *Ceratocystis*. Redhead and Malloch (1977) and von Arx and van der Walt (1987) accepted this argument and commingled yeasts and mycelial taxa in their treatments of the Endomycetales and Ophiostomatales.

Examination of rRNA/rDNA sequence divergence from a limited number of taxa indicated the ascosporogenous yeasts, with the exception of *Schizosaccharomyces*, to form a monophyletic group (clade) distinct from the filamentous species (Barns *et al.* 1991, Bruns *et al.* 1991, Hausner *et al.* 1992, Hendriks *et al.* 1992, Kurtzman 1993a, Nishida and Sugiyama 1993, Walker 1985, Wilmotte *et al.* 1993). Kurtzman and Robnett (1994a) analyzed rRNA sequence divergence from type species of all cultivatable ascomycetous yeasts and yeastlike taxa. This work demonstrated the yeasts, as well as yeastlike genera such as *Ascoidea* and *Cephaloascus*, to comprise a clade sister to the "filamentous" ascomycetes (euascomycetes). *Eremascus*, which forms asci unenclosed in a fruiting body, aligned with the euascomycete clade and may represent a genus close to the phylogenetic demarcation of the hemiascomycetes (excluding *Schizosaccharomyces*) and the euascomycetes. These results substantiate the long-held observation that yeasts cannot be defined solely on the basis of presence or absence of budding. Such members of the yeast clade as *Ascoidea, Ashbya* and *Eremothecium* show no typical budding, whereas *Aureobasidium, Phialophora* and certain other genera of euascomycetes are usually dimorphic. Budding is also a common mode of vegetative reproduction among many basidiomycetous genera. Similarly, vegetative reproduction by fission is shared by *Dipodascus* and *Galactomyces*, members of the yeast clade, as well as by the distantly related genus *Schizosaccharomyces*. Sexual states of all members of the yeast clade are characterized by asci unenclosed in a fruiting body. This feature is shared by only a few taxa outside the yeast clade such as *Eremascus* and *Schizosaccharomyces*. *Myrioconium* and *Trichomonascus* form unenclosed asci but may be euascomycetes as well.

Phylogenetic relationships among the ascomycetous yeasts, calculated from partial sequences of small and large subunit rRNAs, are depicted in Fig. 1. Although there are insufficient phylogenetically informative sites to resolve many of the genera, the comparison gives an overview of relationships. Tree topology is similar to that presented by Wilmotte *et al.* (1993), who examined fewer species, but used nearly complete 18S sequences. In the comparison of partial sequences, most taxa having coenzyme Q with the same number of isoprene units tend to group, but this is not true of the closely related genera *Ashbya, Eremothecium, Holleya* and *Nematospora* which show a variation in coenzyme Q ranging from 5-9 isoprene units. Some congruence is found between location of taxa on the rRNA gene tree and the type of hyphal septal pore produced. The two known genera (*Ambrosiozyma, Hormoascus*) that form dolipore-like septa are closely associated. However, *Arthroascus* produces septa with a simple central pore whereas *Guilliermondella* has multiperforate septa, yet the two genera closely cluster. These incongruities may be resolved by sequencing the complete 18S molecule and by including all known species for each of the genera under study.

rRNA/rDNA sequence comparisons have been quite helpful for understanding species relationships within genera. For example, *Schwanniomyces occidentalis* (Kurtzman and Robnett 1991), *Wingea robertsii* (Kurtzman and Robnett 1994b), and the *Pichia* species, *P. carsonii* and *P. etchellsii* (Yamada *et al.* 1992), were found to be members of the genus *Debaryomyces* on the basis of rRNA relatedness. The initial assignment of these species to other genera resulted from misinterpretation of

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the phylogenetic significance of ascospore morphology. Species originally placed in *Debaryomyces* are characterized by spheroidal ascospores that are roughened by wartlike or ridgelike outgrowths of wall material. An exception is *D. marama* which has ellipsoidal ascospores with wartlike outgrowths and spiral ridges. In contrast, *Schwanniomyces* forms spheroidal ascospores with surface projections and a prominent equatorial ring, *Wingea* has smooth, lenticular ascospores, and the two former *Pichia* species produce smooth, spheroidal ascospores. Furthermore, *Saturnospora* and *Williopsis* both form saturnoid ascospores, yet the two genera are only distantly related (Liu and Kurtzman 1991).

The impact of rRNA/rDNA comparisons on the taxonomy of ascomycetous yeasts is just being felt and will require additional work to fully realize its potential. Major findings to date include: 1) yeasts and yeastlike species are phylogenetically separate from the euascomycetes, 2) the fission yeast genus *Schizosaccharomyces* is phylogenetically distant from the "budding" yeast clade and from the euascomycetes, resulting in the reassignment of the fission yeasts to a separate order, the Schizosaccharomycetales (Eriksson *et al.* 1993, Kurtzman 1993a) and, 3) the demonstration that many phenotypic characters such as ascospore morphology are poor indicators of phylogeny.

Basidiomycetous yeasts

Anamorphic (asexual) basidiomycetous yeasts may be morphologically indistinguishable from anamorphic ascomycetous yeasts. The discovery that the inner cell walls of basidiomycetous yeasts are lamellar when viewed in thin section under the transmission electron microscope, in contrast to the uniform inner layer of ascomycetes, has provided a reliable means for separation of the two taxonomic classes when sexual states are not found (Kreger-van Rij and Veenhuis 1971). A second method of separation, more easily applied, is the Diazonium Blue B (DBB) staining technique (van der Walt and Hopsu-Havu 1976). Colonies of basidiomycetes stain a magenta color in the presence of DBB whereas colonies of ascomycetes remain unstained. From these findings, it has become apparent that basidiomycetes make up a large part of the yeast domain.

Basidiomycetous yeasts (Fell and Kreger-van Rij 1984, Boekhout *et al.* 1993). In the first, teliospores are formed and germinate to produce a basidium that bears basidiospores. This type of sexual cycle shows considerable similarity to the rust and smut fungi. The second type of sexual state lacks teliospores. Basidia develop on hyphae or yeast cells and give rise to basidiospores in a manner similar to the Tremellales (jelly fungi).

Several other characteristics are added to the dichotomy of sexual states. Some taxa produce carotenoids, and the presence of these pigments has been used as a criterion for genus assignment. Ballistoconidia, forcibly ejected vegetative cells, are common to some taxa and their presence is a defining character of genera. Additionally, the hyphal septal pore of basidiomycetous yeasts may be either simple or the ultrastructurally more complex dolipore. Finally, some taxa exhibit the presence of cellular xylose, evidently arising from extracellular polysaccharides (Golubev 1991), whereas other species do not.

Guého *et al.* (1989) presented an overview of the phylogeny of basidiomycetous yeasts from measurements of divergence among partial sequences of large and small subunit rRNAs. Three major groups were resolved; 1) teliospore formers with hyphae having simple septal pores, 2) teliospore formers with hyphae having

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dolipore septa and, 3) non-teliospore formers with hyphae having dolipore septa. On the basis of 18S sequence comparisons, Suh and Sugiyama (1993) placed the smut fungus *Ustilago maydis*, a teliospore former, near the clade comprising representative genera of all basidiomycetous yeasts. In turn, the basidiomycetous yeasts appear to be a sister group to the Agaricales (Berbee and Taylor 1993).

At present, the most extensive phylogenetic comparison of basidiomycetous yeasts is that of Fell *et al.* (1992) who examined 117 species assigned to 23 genera. A 247-nucleotide segment in the D2 region was sequenced; this region resolves closely related species, but may have too few phylogenetically informative sites to accurately assess more distant relationships. The phylogram from that work (Fig. 2) shows the species to be divided between two major clades. The analysis generally supports the concept that taxa assigned to the Tremellales are characterized by dolipore septa and cellular xylose, whereas taxa placed in the Ustilaginales form teliospores, have simple septal pores, and lack cellular xylose. Some exceptions are apparent. The teleomorphic genus *Erythrobasidium* does not form teliospores as do other members of the clade. *Cystofilobasidium* and *Mrakia*, both members of the Tremellales, form teliospores. Another inconsistency concerns the genera located in the lower portion of the Tremellales clade (Fig. 2, B). From what is known of their septal pore structure and lack of cellular xylose, the genera would be expected to group with the Ustilaginales. If their placement is correct, this would suggest that the Tremellales arose from within an already highly diversified group that now represents the Ustilaginales.

Ballistoconidia and carotenoids are found among many genera of the basidiomycetous yeasts suggesting these traits to be ancestral, but not always expressed, thus rendering them of little value for defining taxa. These conclusions were also drawn by Nakase *et al.* (1993). Heterogeneity of coenzyme Q composition occurs in many currently defined genera and will require additional study before its taxonomic significance is fully understood. rRNA sequence analysis demonstrates *Rhodotorula, Sporobolomyces, Cryptococcus*, and *Bensingtonia* to be polyphyletic, further confirming that commonly used phenotypic characters are insufficient for defining anamorphic genera.

A strong start has been made to understand the phylogeny of basidiomycetous yeasts from rRNA/rDNA sequence comparisons, but additional sequencing must be done to better resolve taxa. This will include sequences of greater length as well as inclusion of all known species of a group. For example, the anamorphic genera *Tseuchiyaea* and *Ballistosporomyces*, which were recently defined from differences in partial sequences, may overlap with some earlier described genera (Fig. 2).

Reliability of rRNA/rDNA gene trees to infer phylogeny

With such great emphasis being placed on rRNA/rDNA gene trees to reconstruct phylogenies, it must be asked if there is other evidence to corroborate the conclusions drawn. There are presently only a few comparisons of other molecular sequences that allow this question to be addressed. Sequence analysis of orotidine 5'-monophosphate decarboxylase by Radford (1993) demonstrated the budding yeasts and the euascomycetes to be sister groups as shown from rRNA/rDNA sequences. One unusual aspect of this work was placement of the Mucorales as a sister group to the basidiomycetes. Tsai *et al.* (1994) showed phylogenetic relationships among species of *Epichloë* (Clavicipitaceae) were the same when analyzed from either

rDNA sequences or those from the ß-tubulin gene. Relationships among species of *Dekkera* and its anamorph *Brettanomyces* were essentially identical when analyzed from either nuclear rDNA sequences or from sequences of the mitochondrially encoded cytochrome oxidase subunit II gene (Boekhout *et al.* 1994).

Another aspect of gene tree reliability is the method used for its construction. Most investigators now analyze data using phylogeny inference programs based on cladistic principles. These programs often include a statistics package to test the robustness of competing phylogenetic trees. Several recent reviews address these important issues (Avise 1989, Felsenstein 1988, Hillis *et al.* 1994, Saitou and Imanishi 1989).

Rapid molecular methods for yeast

The specificity of nucleic acid sequences has prompted development of several methods for rapid species identification that are directly applicable to the strains maintained by culture collections. Because these techniques can detect single nucleotide changes, they should first be tested on a large variety of genetically defined strains to understand species variation because aberrant strains may represent different species.

Restriction fragment length polymorphisms of rDNA

rDNAs occur in multiple copies and lend themselves to analysis based on restriction fragment length polymorphisms (RFLP). Magee *et al.* (1987) treated rDNAs from several medically important *Candida* species with a variety of restriction endonucleases and concluded that *Candida guilliermondii, C. tropicalis,* and *C. albicans* produced sufficiently different digestion patterns to allow recognition of each species. Similar results were obtained by Vilgalys and Hester (1990) for several species of the genus *Cryptococcus*. Lachance (1990) used RFLP patterns to map the genetic profiles of 125 isolates of the cactus yeast *Clavispora opuntiae* that had been collected worldwide. Nearly all of the restriction sites that allowed discrimination of individual strains were located in the hypervariable intergenic spacer region.

Data from the preceding studies show that RFLP patterns allow recognition of individual species, as well as individual strains of a species. Consequently, the method has considerable diagnostic value. Estimates of evolutionary relationships from RFLP patterns have been reported for species assigned to *Candida* (Magee *et al.* 1987) and *Cryptococcus* (Vilgalys and Hester 1990). Such estimates would be expected to be less accurate than estimates derived from sequence comparisons because as evolutionary distances increase, the extent of pattern similarities becomes less certain. Bruns *et al.* (1991) listed some of the factors that require attention when using RFLPs.

Random amplified polymorphic DNA (RAPD)

RAPDs represent another methodology that promises widespread application. The technique is based on amplification of genomic DNA in the presence of one or more short (ca. 10-15-mers) oligonucleotide primers of random sequence.

Taxa   Coenzyme Q   Septal Pore

Taxa	Coenzyme Q	Septal Pore
Saccharomyces cerevisiae	6	-
Torulaspora delbrueckii	6	-
Zygosaccharomyces rouxii	6	-
Kluyveromyces wickerhamii	6	-
Pachytichospora transvaalensis	?	-
Arxiozyma telluris	6	-
Issatchenkia orientalis	7	-
Pichia membranaefaciens	7	-
Saturnospora dispora	7	-
Dekkera bruxellensis	9	-
Cyniclomyces guttulatus	?	?
Pachysolen tannophilus	8	-
Citeromyces matritensis	8	-
Saccharomycodes ludwigii	6	-
Hanseniaspora valbyensis	6	-
Nadsonia fulvescens	6	-
Wickerhamia fluorescens	9	-
Ashbya gossypii	6	?
Eremothecium ashbyi	6(7)	?
Holleya sinecauda	9(8)	?
Nematospora coryli	5,6	?
Williopsis saturnus	7	-
Metschnikowia bicuspidata	9	-
Clavispora lusitaniae	8	-
Debaryomyces hansenii	9	-
Wingea robertsii	9	-
Cephaloascus fragrans	9	Cent
Ascoidea africana	?	?
Saccharomycopsis fibuligera	8	Multi
Arthroascus javanensis	8	Cent
Guilliermondella selenospora	8	Multi
Botryoascus synnaedendrus	8	Multi
Hormoascus platypodis	7	Doli
Ambrosiozyma monospora	7	Doli
Lodderomyces elongisporus	9	-
Dipodascus albidus	9	?
Galactomyces geotrichum	?	?
Sporopachydermia lactativora	?	-
Stephanoascus ciferrii	9	Multi ?
Zygoascus hellenicus	9	Cent
Yarrowia lipolytica	9	Cent
Wickerhamiella domercqiae	?	-
Dipodascopsis uninucleata	?	Cent
Lipomyces starkeyi	9	-
Zygozyma oliphaga	8	-
Eremascus fertilis	?	Cent
Emericella nidulans	?	Cent
Ceratocystis fimbriata	?	Cent
Protomyces inundatus	?	?
Taphrina deformans	10	?
Schizosaccharomyces pombe	10	-
Filobasidiella neoformans	10	Doli

Fig. 1. A phylogenetic tree derived from maximum parsimony analysis depicting the ascomycetous yeasts, yeastlike fungi, and various reference species. The phylogram was calculated from combined small and large subunit rRNA partial sequences as described by Kurtzman and Robnett (1994a). Branch lengths are proportional to nucleotide differences, and the numbers given on branches are the percentage of frequencies with which a given branch appeared in 100 bootstrap replications. Branches without numbers had frequencies of less than 50%. Coenzyme Q data are from the compilation of Barnett et al. (1990) and refer to the number of isoprene units in the sidechain on the parent molecule. Information on septal pores is from Kreger-van Rij and Kurtzman (1984) and Barnett et al. (1990).
Cent = central pore, Multi = multiperforate septum, Doli = dolipore-like.

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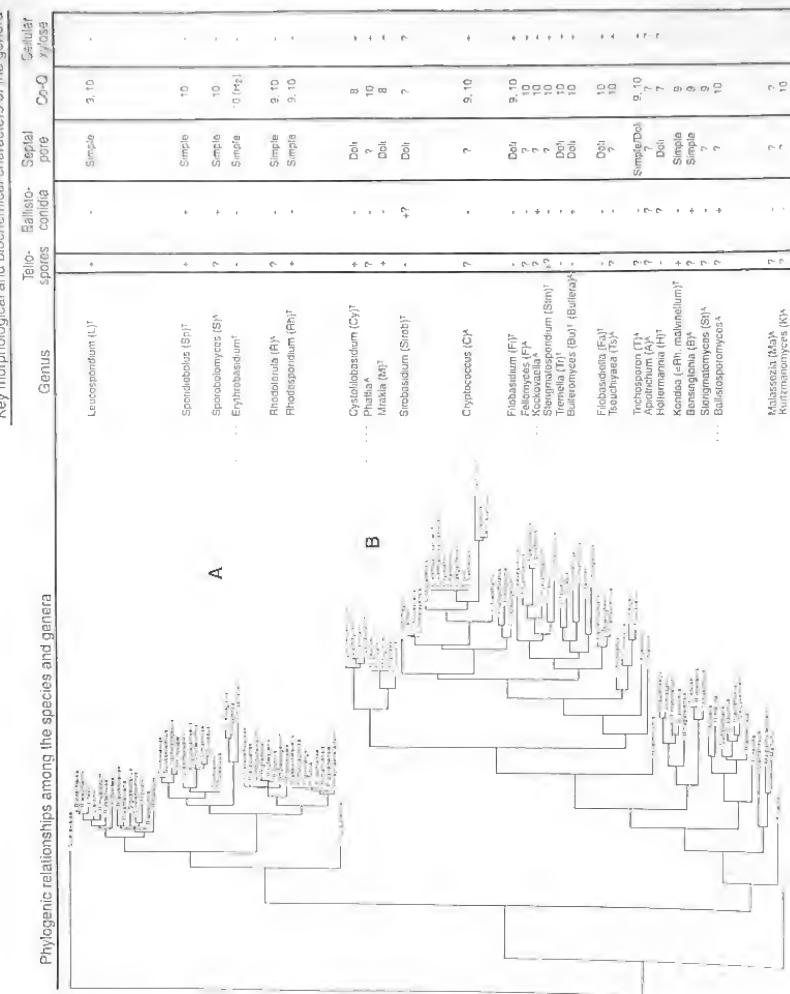
Fig. 2. A phylogenetic tree derived from maximum parsimony analysis depicting the basidiomycetous yeasts. The phylogram, which represents the most parsimonious tree, was calculated from rRNA sequences of the D2 region of the large subunit as described by Fell *et al.* (1992). Branch lengths are proportional to the number of nucleotide differences. Bootstrap values were not determined. Clade A is comprised of taxa placed in the Ustilaginales. Taxa in Clade B are predominantly assigned to the Tremellales, but see text for discussion. The strain of the outlying species *Rhodotorula buffonii* that was examined was shown to be a misidentified ascomycete. Genera preceded by a broken line were not in the original analysis and have been placed in the present phylogram on the basis of other studies (Guého *et al.* 1989, Nakase *et al.* 1993,

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The amplified products are visualized on an agarose gel and strains identified from matching band patterns. Hadrys *et al.* (1992) discussed details of this procedure and also noted points of technical difficulty.

Species-specific PCR reactions

Peterson and Kurtzman (1991) and Kurtzman (1993b) have shown that the 5' end of the large subunit rRNA/rDNA is sufficiently variable to detect even closely related yeast species. Fell (1993) used this region to identify individual yeast species by applying a three-primer, PCR-based technique. In this method, the reaction mix includes genomic DNA, two external primers for the D1/D2 region of large subunit rDNA, and a species-specific internal primer. The external primers allow amplification of the ca. 600-nucleotide D1/D2 region, but in the presence of a species-specific primer (third primer) the amplification product is shorter and easily detected on an agarose gel. There is currently considerable activity in the field of molecular probe development and rapid methods appear nearly ready for widespread use.

Future prospects

rRNA/rDNA sequence comparisons have provided us with a genetic perspective to the relationships among yeasts and other fungi. On the basis of limited comparisons, rRNA/rDNA gene trees are generally congruent with phylogenies from other molecular sequences, but additional studies should be done to verify this preliminary assessment. In many cases, molecular comparisons are supportive of relationships among the fungi determined from traditional morphological studies. In other cases, the molecules have led us to new perspectives. One of the greatest challenges facing the field of mycology is to use molecular data to develop a phylogenetic system of classification.

The unique nature of molecular sequences promises to allow development of species-specific molecular probes for rapid strain identification. Once this technology becomes widespread, a realistic assessment of the earth's biodiversity may finally be possible.

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PHYLOGENY OF FILAMENTOUS ASCOMYCETES DEDUCED FROM ANALYSIS OF MOLECULAR CHARACTERS: PRESENT AND FUTURE

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Abstract. Phylogenetic studies of ascomycetes dispersed by arthropods have benefited from the use of rDNA sequence analysis. At higher taxonomic levels it has been necessary to compare a variety of taxa that either lack corresponding morphological characters (*Raffaelea*, *Ambrosiella*, *Laboulbeniales*, yeasts) or have characters that may be the result of convergent evolution (*Ceratocystis*, *Ophiostoma*, *Pyxidiophora*). For example *Ambrosiella* has been found to be polyphyletic with one group of species related to *Ceratocystis* and the other group allied with *Ophiostoma*. Species of *Ambrosiella* are asexual and could not have been compared to sexual forms soley on the basis of morphology. Several genera with evanescent asci and arthropod associations previously have been placed in *Ophiostomatales*. We now know that *Ophiostoma*, *Ceratocystis*, and *Pyxidiophora* are not closely related, and, in fact, at least six lineages of such fungi have been identified. One other taxonomically problematical group, *Laboulbeniales*, lacks a number of morphological characters that can be compared with filamentous forms (including even the hyphal filaments!). The hypothesis of a relationship with *Pyxidiophora* based on few morphological characters now has support from rDNA sequence analysis. It is important to note that our results could not have been obtained so easily were it not for the previous studies of the morphology, life histories, and associations of the fungi. For the future it will be important for mycologists in distant localities using different techniques to cooperate in discovering and classifying the Earth's fungal diversity. In addition to the continuing development of phylogenetic techniques, another inovation is important. Worldwide collaboration already is enhanced at the close of the twentieth century by our ability to communicate rapidly and at will using computerized communication networks, and this technology must be available to all mycologists throughout the world.

Previous attempts at phylogenetic classification of ascomycetes have been confounded by the presence of convergent morphological features, the absence of characters that extend across all taxa, and an inability to determine character polarity. However, a non-morphological approach, specifically ribosomal DNA (rDNA) sequence analysis has provided new characters that circumvent the problems assciated with the use of morphological characters.

In our work with arthropod-associated fungi we have been particularly interested in the ascomycetes with long necked perithecia and evanescent asci and the possibility that these characters are the result of convergent evolution to facilitate ascospore dispersal by arthropods. Some of this work has been published and will be reviewed only briefly to provide an example of the usefulness of the technique. In this case nuclear-encoded rDNA sequence was applied to the study of ascomycetes previously placed in *Ophiostomatales*, or in some cases, never placed in an ordinal level taxon. The taxa are all characterized by evanescent asci and, in most cases, are arthropod dispersed.

The relationships hypothesized on the basis of rDNA analysis indicate that a divergence of the fungal lineage gave rise to the basidiomycetes and ascomycetes. Both groups presumably arose from a filamentous ancestor. An early ascomycete divergence, or perhaps several, produced such forms as *Taphrina*, *Schizosaccharomyces*, *Protomyces*, and *Pneumocystis* (Sugiyama and Nishida 1993, Nishida and Sugiyama 1994, Kurtzman 1993). These fungi are marked by yeast-like stages as well as mycelial growth. It is interesting that they often have been at the center of taxonomic controversy and all have been considered basidiomycetes at

some time in their history.

The next major divergence of ascomycetes gave rise to two lineages, the yeasts of *Saccharomycetales* and the filamentous ascomycetes. It is these two groups that have numerous arthropod associations. In addition some of the species in both groups have morphological and biochemical characters in common. For example the galeate or hat-shaped ascopore morphology, yeast thallus, and various conidial stages found among species of *Ophiostoma* and *Ceratocystis* as well as some yeasts led to the appealing hypothesis that these forms might constitute a monophyletic group (Redhead and Malloch 1977, von Arx and van der Walt 1987). With most of the major groups of yeasts examined, it does not seem likely that any of them are derived from the filamentous ascomycetes, and the characters typical of arthropod-associated ascomycetes are convergent features.

One major accomplishment involving the filamentous ascomycetes has been the resolution of the relationship of *Ceratocystis* and *Ophiostoma*. At times species of both genera have been considered congeneric. We now have evidence that not only are the two genera distinct, but also they occur within different subclades of perithecial ascomycetes (Spatafora and Blackwell 1993). Other filamentous forms with evanescent asci including *Pyxidiophora*, *Kathistes*, and *Subbaromyces* lie outside of the perithecial ascomycetes, and each may be part of an independent lineage. The yeast-like *Symbiotaphrina kochii* also is in this part of the tree.

Several years ago Dr. David Malloch and I (Blackwell and Malloch 1989) used morphology and life cycle studies to propose a relationship between *Pyxidiophora* and *Laboulbeniales*. This hypothesis was tested using rDNA analysis and the evidence supports the previous conclusion of their close relationship (Blackwell 1994). However, the nearest relatives of the *Pyxidiophora-Laboulbeniales*, *Kathistes*, *Subbaromyces*, and *Symbiotaphrina* lineages have not been identified because this part of the tree is unstable. Clarification awaits addition of more taxa from the discomycetes and loculoascomycetes, and, perhaps sequencing of other DNA regions.

Our more recent work on arthropod-associated ascomycetes involves the analysis of more rapidly evolving gene regions to develop species concepts for *Pyxidiophora* and the asexual taxa associated with *Ceratocystis* and *Ophiostoma*. Of particular interest is the finding that *Ambrosiella* is not a monophyletic group, but consists of two groups of species, one related to *Ceratocystis* and the other allied with *Ophiostoma*, both in association with scolytid beetles. Convergence of conidial forms has followed the pattern of convergence in their sexual relatives (Cassar 1993). Another asexual lineage does appear to be monophyletic. Species of *Raffaelea*, however, have diverged from an ophiostomatalean lineage as associates of platypodid beetles.

I have discussed examples of the impact of recent technological advances in helping to define monophyletic groups and clarify the phylogenetic relationships of the ascomycetes. But there are other reasons why mycologists study fungi, and the same methods are useful in these cases as well. Molecular technology has become important to us in identifying particular fungi at a variety of taxonomic levels. From the practical point of view, a knowledge of species that attack plants and animals is necessary. Not only is an identification of species necessary, but it also has become important to identify populations among species of some fungi.
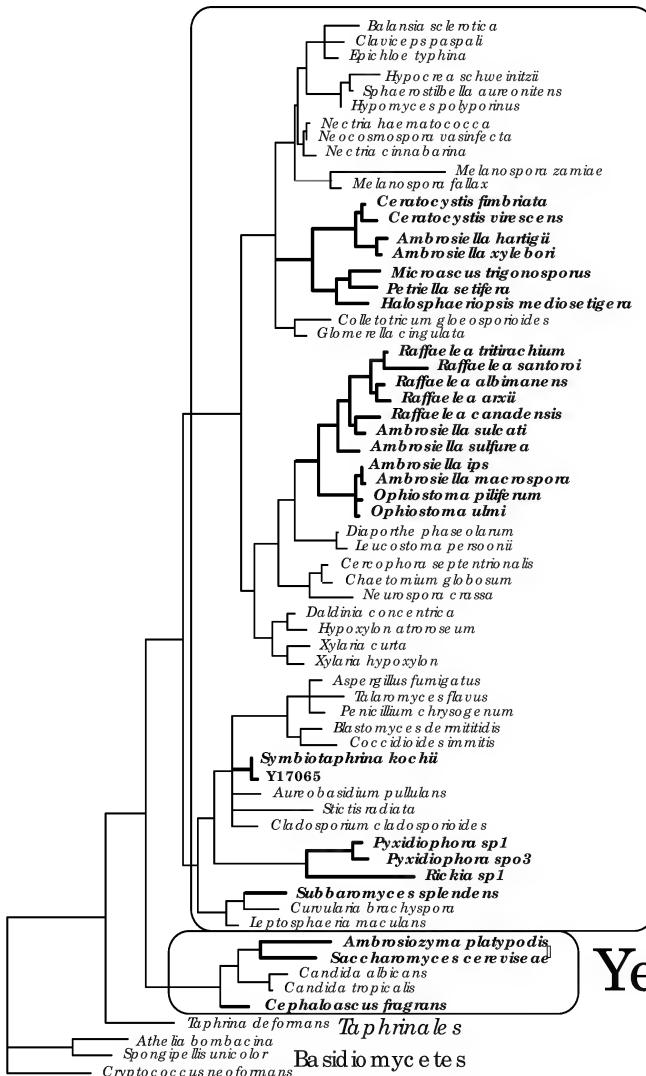
Fig. 1. Phylogenetic hypothesis of the relationships of certain arthropod-associated ascomycetes based on analysis of small subunit rDNA partial sequences using parsimony criteria. See Spatafora and Blackwell (1993) and Blackwell (1994) for details.

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The identification of ectomycorrhizal fungi usually has depended on collection of basidiocarps during seasonal fruiting periods to answer questions about the presence and prevelance of individual mycorrhizal species. However, it now is possible to use molecular methods to identify the fungi to family and, sometimes to species, from samples of field collected roots at any time of the year (Bruns and Gardes 1993). The technique already has shown that the most important mycorrhizal species in terms of frequency are not those that are discovered to be common on the basis of fruiting. The tools that are being developed soon will allow mycologists to determine the precise ecological relationships of the symbioses.

Molecular techniques applied to population studies of *Phyphthophtora infestans* (Drenth *et al.* 1993, Therrien *et al.* 1993), *Ophiostoma ulmi* (Brasier 1994), and *Cryphonectria parasitica* (Anagnostakis 1993) are helping to determine the origin and monitor the epidemiology of introduced pathogens, to determine the importance of different modes of reproduction in the development of aggressive populations and, perhaps, to discover the basis of speciation. All of this information depends upon the use of molecular techniques to provide more characters than mycologists have ever had available to them. However, in addition the characters are available at all taxonomic levels, extend across all taxa, and can be polarized.

The polymerase chain reaction (PCR) has lead to rapid progress in molecular methods. As the technology is simplified through continued improvements in automated DNA sequencers and more biologists can use the techniques, the associated costs will come down. In fact there are now rumors of automated sequences that will become available within a few years that will cost no more than a sophisticated light microscope.

It is important to recognize that our new-found ability as mycologists to address problems that previously eluded us, should be viewed as contributing to a worldwide revitalization of fungal studies that is even greater than the electron microscopy revolution of the 1960's and 1970's. It also is possible to use morphological characters with new confidence because their polarity and convergent nature can be inferred with the help of molecular characters. If one looks at previous phylogenetic hypothesis the inability to determine polarity and recognize the possibility of convergence has most often caused mistaken interpretations.

It is obvious to me that mycology is poised to make progress in all areas. Previous morphological studies have contributed to the basic body of knowledge that provides the context for molecular applications, and they will continue to be important in the discovery of the great fungal diversity (Hawksworth 1991). The importance of isolation and culture is increased by the knowledge of the genetic potential of diversity. In these endeavors all mycologists do not have to use all techniques. Some may not have the inclination or the technology to apply molecular methods. Those versed in molecular techniques may not have the means of acquiring the training in morphology that may require years of concentrated study. However, teams of mycologists with different backgrounds must cooperate, perhaps in formally organized groups in distant geographical regions. These collaborations already are practical because of another technological advance, the rapid communication available through computer communications networks. It is imperative that the mycologists of the world be united by "e-mail" before the end of the twentieth century so that all may participate fully in the task at hand.

Acknowledgements

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IMPORTANCE OF MORPHOLOGICAL AND ANATOMICAL CHARACTERS IN FUNGAL TAXONOMY

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Introduction

Morphological and anatomical characters and their importance to the taxonomy of fungi must be seen in a historical context. The reason is obvious: when taxonomic classification started only macroscopical characters were taken into account (as it was the case for the study of other organisms). Later anatomical and microscopical characters were added as the science continued to develop. Today, physiology, molecular biology, genetics, enzymology, and ecology all contribute to an understanding of living organisms and their classification.

With this background in mind, I will discuss the following questions:

1. How has our knowledge at the most "basic level" of "species", the level used in taxonomy, evolved; and how important are morphology and anatomy in this conception?

2. How has "taxonomy" been interpreted over time?

3. What is the currrent situation, with an emphasis on morphology and anatomy?

4. Is the importance of morphology and anatomy similar for all groups of fungi?

5. With respect to this symposium's emphasis on tropical mycology: is the situation in the tropics different from the one in temperate regions?

How has our knowledge at the most "basic level" of "species", the level used in taxonomy, evolved; and how important are morphology and anatomy in this conception?

The often very sudden appearance on fruit-trees of larger fungi as well as the immediate impact of moulds on the everyday lives of human beings prompted the initial study of fungi. To commence such a study, however, it was necessary to begin the process of classifications.

In the case of fungi, the first observations were visual. This is apparent given that the eating of macrofungi was important. As such, the first classifications were very pragmatic. Classifying was done on the basis of edibility and morphology in the very broad sense of the word.

It is to be expected then that the oldest classification, Clusius (1601), which made the distinction between *Fungi esculenti* and *Fungi noxii et perniciosi* would be adopted by Bauhin (1623) and Van Sterbeeck (1675) who also made use of these criteria. Ray (1686-1704) added the morphological criterion of whether or not the fungal fruitbodies were lamellate, as well as other ecological criteria. Magnol (1689) continued in this line, though Dillenius (1718) added a phenological criterion, which was subsequently refined by Micheli (1729).

Linaeus developed his system of classification on the grounds that (Micheli's) system was not satisfactory. Gleditsch (1753) introduced, in turn, more morphological characters, while Person (1794 and following) made a classification

system based on gross morphology that included very accurate descriptions of serveral common microfungi. The most important contribution was made by Fries who described 1860 species of *agarics* in twenty genera, using mainly macroscopical characters.

I could go on, but that is not the aim. The important thing to remember is that descriptions and classification schemes started as very empirical. From this simple beginning, gross morphology was introduced and shortly thereafter microscopy was added. In the beginning, microscopy was very basic, but it progessed to finer microscopy. Later, developmental characters were introduced. Later still, the results from experimental biology, ecology, physiology, and biochemistry were included as characters for classifying fungi.

Let us look at the actual situation. I have selected a few characters from recent publications, selecting them from different systematic groups. As a general conclusion, it must be said that it was rather difficult to find descriptions of new species which used characters other than morphology and anatomy. This situation is easy to observe through a short analysis of vols. 46, 47, and 48 of *Mycotaxon*. Only systematic treatments have been taken into account, and lichens were not included.

Volume	46	47	48
Basidiomycetes			
morphology, anatomy, ecology:	8	10	10
morphology, anatomy, culture:	-	-	1
morphology, anantomy, experimental:	-	-	1
numeric:	-	-	1
Ascomycetes and deuteromycetes			
morphology, anatomy:	13	12	2
morphology, anatomy, culture:	14	5	12
morphology, anatomy, culture, developmental:	3	4	3
morphology, anatomy, culture, physiology:	-	-	1
morphology, anatomy, culture, genetics:	-	1	1
morphology, anatomy, culture, computer:	-	2	-

It is obvious that even in today's practices morpholgy and anatomy remain the basic characters in systematic research and taxonomy.

I did the same for *Mycological Research,* but as the bulk of the work is experimental and not taxonomic, this exercise was not very valuable. Nevertheless, it is clear that while *Mycological Research* explores many new fields, its studies do not influence basic taxonomic research to a great extent. These fields of study are excellent, however, as they force taxonimists to think about biological phenomenon which they will have to account for eventually.

Descriptions and the delimitation of taxa remain very traditional and are based primarily on morphology and anatomy. Description, however, demonstrate an increasing degree of precision. The increased precision in describing morphological and anatomical characters can be assessed easily by examining the introduction of Singer's *Agaricales* in modern taxonomy (1975) or Kuhner's *Les Hymenomycètes agaricoïdes* (1980), for example.

Within this context it should be stressed that often small differences in morphological or anatomical characters can be extrememly important for understanding the biology of a given species. Good examples can be found in experimental phytopathological work where it has been demonstrated that spore form and spore dimension play a major role in the place where a spore will land on a phanerogam in order to infect its host.

Still, morphology and anatomy will continue to play a major role in systematics because our knowledge, gathered from previous generations, has created a framework into which more recent observations are to be fitted. Such a framework put together by so-called modern techniques is lacking for a large number of species. Given the enormous investment in human and other resources needed to produce this framework, it cannot be expected that this situatiuon will change very fast. Still, the results from new techniques are very welcome. Quite often these recent inquiries derive from studies of species or species groups that are easy to cultivate or to collect, or from studies of fungi of high economic value.

How has "taxonomy" been interpreted over time?

Classification and taxonomy aim to order certain kinds of information about living organisms. Whereas, on the whole, species and infraspecific categories can be defined by the existence of the interbreeding groups of individuals, this is no longer the case for the supraspecific categories used in taxonomy.

Genera and more universally supraspecific categories can be approached in different ways:
- genera (and higher taxa) can be recognized in virtue of being objectively recognisable in nature from patterns of morphological similarity (Kornet, 1994: 5);
- genera exist in virtue of there being a historical entity, possessing cohesion over time as assured by relationships among its members (Kornet, 1994: 5).

In early classification systems, morphological and anatomical characters were absolutely predominant in ordering information about living organisms: phenetic similarity was the basis of these classification schemes. At that time, however, it was often impossible to eliminate all background considerations in the classification which were due to convergent or parallel evolution.

With the introduction of cladistic methods, this situation changed to a certain extent. However, one of the major obstacles in using cladistic methods is the lack of knowledge of most of the characters of organisms. In most instances the characters described in the past were those which were considered as taxonomically important, and it is these characters which have noted by ("phenetic") taxonomists.

What is the currrent situation, with an emphasis on morphology and anatomy?

From a theroretical point of view, a phylogenetic methodology can give far superior classifications than the phenetic approach. On the other hand, when looking at the results of cladistic analyses in mycology, it is obvious that the interpretations of the cladograms have been leading to interpretations which are cleary ambiguous. This lack of clarity is partly due to the characters which were readily employed in the studies.

In order to address this ambiguity, then, if the group of species for which the analysis will be made has not been treated monographically, data should be included from a number of sources: different periods, different authors and different taxonomic traditions. And yet, as can be seen in even recent monographs, it can be difficult or even impossible to construct a solid data matrix as, for instance, phenetic taxonomists have preselected certain characters in their descriptions on the basis of their experience with the group. What this practical consideration highlights is that taxonomists can easily prejudice their analyses, if they do not assess their taxonomic characters adequately enough.

Taxonomist must use good descriptive terminology consistently, describing character states very precisely. Given the examples I have seen, I must stress that the collecting of basic data needs to be done more rigourously.

Going through the recent cladistic mycological literature, I have noticed that too often character sets have been accummulated from the literature without any real expertise in the group, which has led to the misinterpretation of characters and their significance. Unfortunately, the results from such analyses cannot be expected to improve our knowledge of systematics.

To avoid these sorts of problems from a practical point of view means that ecologists, phytopathologists, medical mycologists as well as amateur mycologists must have a common understanding of characters at the species level. Thus far, experience shows that easily observable morphological and anatomical characters have so far been the most readily available.

Is the importance of morphology and anatomy similar in all groups of fungi?

Considering fungi as a whole, it is obvious that classification and systematics have employed different starting philosophies. As a result, it can be expected that systematics developed along different lines in the case of yeasts and yeast-like fungi as compared to other groups of fungi.

The origin of systematics of yeasts and yeast-like fungi was in the first place an applied study, having its roots in the fermentation industry. From its practical point of view, selection of physiological properties was the most important character. As systematics progressed along these lines, a new study developed which we now know as bacteriology. In this new field, the use of morphological characters was hardly possible because of the lack of a well-differentiated thallus that would have been useful for distinguishing between groups of bacteria.

The systematics of the majority of fungi developed along the line of practice of systematics for higher plants. They began as primarily morphological and progressed to include anatomical characters. For a long time, systematic of both "types" of fungi developed on these lines, and it was thought that yeasts were a heterogeneous groups of organisms. But with the introduction of new techniques, links were made between both groups when it became obvious that yeasts were unicellular, ontogenetic stages of ascomycetes and basidiomycetes.

From this perspective, it is obvious that for the classification of yeasts and yeast-like fungi an important contribution will be made by mycologists who are acquainted with systematics of ascomycetes, basidiomycetes, endomycetes and ustomycetes. One result may be that the virtual non-exception to the general rule that classification is primarily based on morphology and anatomy will disappear. However, from a

practical point of view, the artificial classification of yeasts and yeast-like fungi will continue to be valuable as an approach for specific purposes, though not as a general approach for basic systematics.

With respect to this symposium's emphasis on tropical mycology: is the situation in the tropics different from the one in temperate regions?

Basic knowledge of fungi from the tropics is extremely poor given the large number of species which require describing. On the other hand, this situation can be advantageous as mycologists can, by adapting their experience from temperate regions, collect and describe fungi from the tropics according to the specific requirements of new techniques, such as cladistics. Furthermore, since tropical mycology is not as encumbered by nomenclatorial problems as mycology conducted in temperate regions, this circumstance can be considered as an advantage. Systematic mycologists can profit from this situation, though they need to be as careful and consistent as possible when collecting and describing specimens. The need for care is certainly the case where, because new techniques are more limited in the tropics, systematics will continue to rely on morphology and anatomy.

Conclusion

Morphology and anatomy have been the primary characters used in the systematics of fungi. Other types of characters which have been considered outmoded have not always been adapted by taxonomists. At present, taxonomists have high performance computers at their disposal, which have greatly enhanced the possibility for networking and the exchange of data and results between scientists. Even though taxonomy has evolved considerably from its early beginnings, it is still very important that it continues to develop, to adapt new techniques and technologies as they arise. The critical issue is that taxonomy remains a central discipline in the study of fungi. This possibility is especially significant in view of the fact that systematists work to synthesize everything that is known about organisms. For it is through these syntheses that more complete schemes of relationships can be developed. Though there is a distinct possibility that the importance of morphology and anatomy will diminish with the development of these syntheses, from a practical point of view, and this is especially true for tropical mycology, they will remain the most important characters for separating adjacent taxa.

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POSSIBLE PROGRESS OF MORPHOLOGICAL ANALYSIS IN FULGAL TAXONOMY

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Introduction

From its beginning, fungal taxonomy was based on morphology, first by means of direct examination of the fungus and later by microscopical observations. Since the launching of the new techniques by the pioneer microscopists of the 17th Century such as Hook and Leeuwenhoek, optical microscopy progressed considerably to the end of the 19th Century, when the cultural study of fungi was introduced. This new perspective opened the possibility of a complementary developmental approach to fungi.

Much later, around 1950, the use of the Transmission Electron Microscopy enabled researchers to make comprehensive progress in the study of fungal structure and ontogeny, while the use of Scanning Electron Microscoopy permitted more detailed surface and three-dimensional observation of fungal specimens. Generally speaking, data obtained by electron mircoscopy (Pegler and Young 1971, Cole and Samson 1979) did not impair those obtained by good optical observations and descriptions, rather it improved them. Accordingly, both macroscopical and microscopical approaches have become increasingly useful and complementary.

Still later in the 1980's, the advent of molecular techniques was applied to the taxonomy of fungi. This development gave raise to the belief that the descriptive phase of taxonomy was drawing to a close (Hillis 1987). Nevertheless, it is becoming more evident that these new techniques do not modify substantially the results of traditional morphological taxonomy. Rather these techniques help to clarify questions suggested by the limited resolution of morphological studies, such as for example, the exact delimitation of taxa or the definition of holomorphic relationships (Kohn 1992). In point, it is important to stress that these various approaches should be considered as complementary and not exclusive of one another. As such, microscopists should be encouraged to assist molecular biologists in their efforts to order any new data in the context of taxonomy, and vice-versa (Reynolds and Taylor 1993).

In this respect, while morphological analysis may be understood to lie at one "end"of a phyllum, it remains still in a process of evolution. Given that such techniques will continue to evolve, two axes need to be considered:

1. the improvement and simplification of observational techniques
2. the interpretation of multiple data sets

The improvement and simplification of observational techniques

At present, morphological studies are generally performed simultaneously through direct observation *in situ* through optical and electron microscopical examination. Of course, each technique has its limits, its advantages and

disadvantages. The performance and efficiency of optical microscopy does not need to be demonstrated. Still, it is a stumbling block for fungal identification. Although it is relatively inexpensive and thus readily available in all countries as well as easy to use, magnification (x 2000) and resolution (0.2 μm) can be limiting factors despite the efficiency of this device.

Electron microscopy, on the other hand, permits high magnification (x 100000) and resolution (3-10 Åm), but both transmission and scanning electron microscopes necessitate observation under vacuum conditions which requires special preparation of specimens (fixation, dehydration, sectioning, and staining). Recently, considerable progress has been made in these areas particularly with the development of low temperature fixations (Hock 1986). A low temperature scanning microscope (LTSEM) is now widely available and allows for morphological observation in hydrated states (Jeffree and Read, 1991).

Even if these various forms of electron microscopy are really useful for fungal taxonomists, however, these techniques require heavy and expensive equipment which is not always available to everyone in all parts of the world. An ideal situation for the future would be the development of less expensive equipment that allows an easy observation of the specimen at high magnification and resolution but does not require that the specimen undergo preparation, like the use of vacuum.

New techniques are progressing in this direction. The most promising for morphological analysis in fungal taxonomy is Confocal Microscopy. It bridges the gap betweeen conventional optical and electron microscopes. (Laurent *et al.* 1992, Shotton 1989). In this case, the image is produced by scanning a diffraction limited laser over the specimen, exposing an extremely small volume of it to the incident light, and thereby restricting sources of reflected light. The most novel imaging mode accounts for vertical sectioning. Instead of scanning the specimen along the x-y planes, this mode generates images by scanning optical sections that are parallel to the axis of the microscope. This approach has the advantage of limiting out of focus information from the observed image.

Confocal microscopy does not need any preparation of the specimen except for fluorescent staining. With resolution down to 0.25 μm, the technique is used frequently in cytology, cytometry, and molecular biology (Shotton 1989). Preliminary results of morphological observation obtained with *Penicilium chryogenum* (Fig. 1) shows clearly the absence of out of focus blur in the confocal images. These results also show the fine resolution in successive vertical planes which allows for the exploration of thick structures (70-80 Åm).

Cell surfaces can also be investigated by other new kinds of microscopes: the Scanning Probe and Scanning Tunnelling Microscopes (STM) as well as the Atomic Force Microscope (AFM) (Guckenberger and Baumeister 1992). Research with STM is still focused on exploring type of experiments that might be more feasible in the future. At present, it is being tested principally on non-biological materials. In contrast, AFM has already been used quite successfully for obtaining images of biological specimens in aqueous solutions (Butt *et al.* 1990, Kasas 1992). The principle technique applied in AFM involves scanning the surface of the specimen with the tip of a very thin probe (diamond or silicium). The three dimensional scanned displacements are recorded and analysed by a computer. AFM combines a high resolution (80 Åm on blood cells) without the need for sample preparation as it works in aqueous environments.
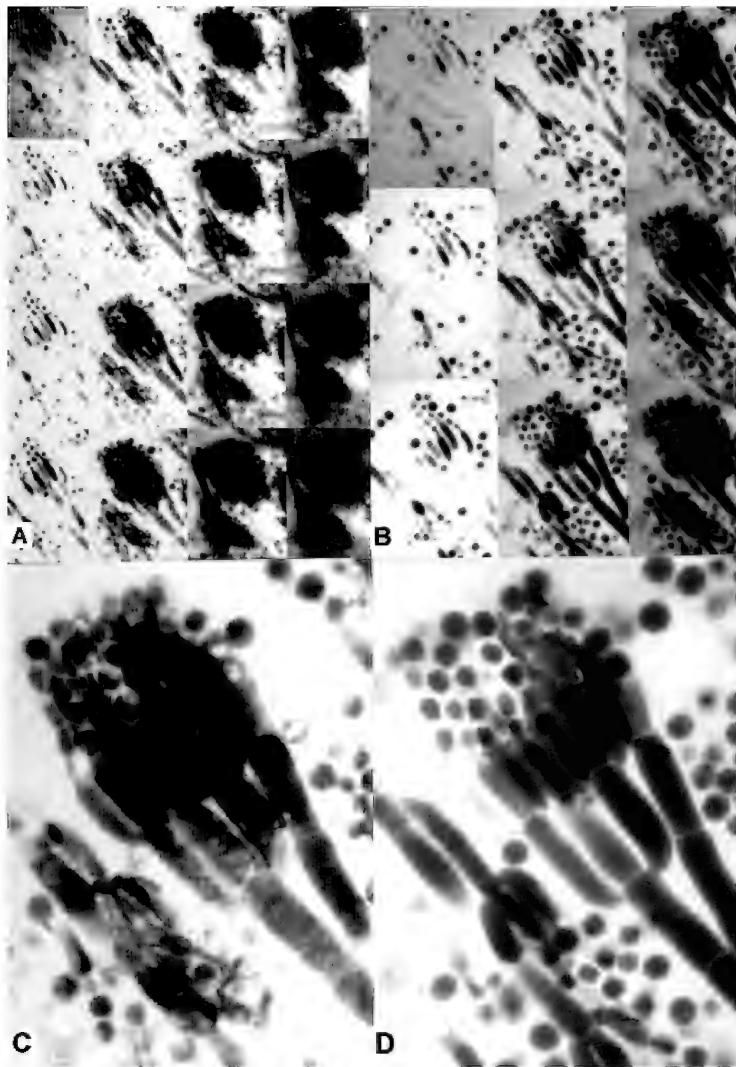
Figure 1. *Penicillium chrysogenum* Thom, Coloration: Fluorescein-dextran (negative coloration). Confocal Microscope Biorad MRC 1000. A-B: Optical sections at different focal planes separated by 1 Ám. A: Non-confocal, B: Confocal optics. C-D: Detail of penicillus. C: Non-confocal, D: Confocal optics.

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It is currently used for studying molecules (proteins, phospholipids, and DNA) constituting organic matter. The advantage of the technique is that it permits observations in environmental conditions with good magnification and resolution. The lightness of this equipment also constitutes an advantage.

Even if these new microscopy tehniques are still in the research phase, we can safely assume that these techniques, in particular confocal and atomic force microscopy, will be used in the near future for fungal morphological observation and taxonomy.

The interpretation of multiple data sets

To be effective in taxonomy, observations must be intrepeted and represented as objectively as possible. Until now, line drawings and photographs have been very effective for providing clear visual representations of macro- and microscopical observations, but because they are man-made and interpretative, they are often very expressive and artistic, and thus quite subjective.

On the other hand, quantitative morphological analysis provides a means to translate visually apparent structures into numerical data that can be subjected to the powerful methodologies of mathematical analysis. (Wagener 1990, Steer 1991). Because of its capacity for decomposing and anaylsing a great number of images, image analysis may be a very effective tool for limiting subjective interpretations. The important point is to obtain the maximum information from visual images so that the data can be interpreted with the greatest possible confidence. This possibility may also be very helpful for taking into account the variability of specimens. One of the advantages of the technique is that image analysers can be integrated directly into the apparatus of microscope as they are already in confocal and scanning probe microscopes.

In addition to image analysis, another way to exploit a large number of morphological observations may be the use of morphometrics. The field of morphometrics is traditionally concerned with methods for the description and statistical analysis of shape variation within and among samples of organisms adjusted to a common size, measuring certain landmarks of an organism (length, width, etc.). An example of the impact that morphometric analysis can have on fungal taxonomy is evident in the recent publication by Vanev (1993) on the conidia of 14 type specimens of *Discosia* section *Strobilina*. Analyses of the shape as well as the length and width measurements of the conidia enabled the author to reach decisions about the taxonomic status of the specimens, that is, to gather data useful for separating and grouping taxa.

Many methods agree on the nature of the differences among a set of shapes, but fewer agree on the relative significance of the differences between these shapes. A new trend in morphometrics is to incorporate data on the geometry of the structure so as to distinguish changes due to mere differences of size from those which describe purely homogeneous features (Zelditch *et al.* 1992). The ideal aim would be to eliminate from the analysis, dimensional features that are due to the natural variability of biological material, so that phenetic or cladistic relationships can be estimated more accurately.

Conclusion

Interesting progress is being made in the morphological observation and analysis of biological material. The methods and equipments used in these techniques are being simplified considerably without loss of performance. Even though the analysis and the objective interpretation of data remain at the research phase, in part because of the variety of biological material, great possibilities exist for these techniques as they are effectively adapted to fungal taxonomy. These technical and analytic approaches seem promising for the progress of fungal taxonomy, as they also are an interesting subject for research in themselves.

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MYCOFLORISTIC STUDIES IN SOUTH CENTRAL AFRICA: STATUS, CONSTRAINTS, OPPORTUNITIES AND STATEGIES

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Abstract. Mycofloristic studies of the South Central African region, comprising Malawi, Zambia and Zimbabwe, have largely focussed on the identification of macrofungi, apart from fungi of agricultural importance. The only relatively well studied group is the Aphyllophorales, with over 200 known species. In addition to their studies on the Aphyllophorales, visiting scientists have contributed immensely to our present knowledge of the Agaricales, Boletales, Cantharellales, Russullales and other non-basidiomycete fungi. *Termitomyces*, *Cantharellus* species, edible boletes and species of *Russula* and *Lactarius* have a unique contribution to the diets of many people during the rainy season. This has prompted publication of country and regional guides to mushroom identification and mushroom poisoning. Most studies of the larger fungi have been of a taxonomic nature, though mushroom productivity studies have just begun. The major taxonomic approach has stressed morphological and anatomical characters; supplemented by chemical, ecological, cultural and other characteristics. We find the approach to be pragmatic under our circumstances, and applicable to a large range of fungi, though its shortcomings have been realised in recent collaborative studies on African *Armillaria*. The genetic basis of species differentiation has largely been of an academic interest. Unfortunately, taxonomic studies are still regarded as being peripheral to agricultural and forest pathology/mycology and most identifications of fungi are conducted by institutions outside Africa. There is a lack of qualified scientists, as well as adequate and continuous funding at some established research institutions. There is need to undertake more fungal inventories and ecological studies because of their importance to aspects of the conservation of fungal and ecosystem biodiversity. Furthermore, with an estimated annual deforestation rate of over 250 000 hectares in the region, some species will eventually be lost before they are known. Assistance in training local scientists must be sought, and there should be a provision for suitable equipment and literature. Local, regional and international support for such activities should be mobilised. The importance of herbaria for educational, reference, conservation and other purposes should be publicised. These are our present challenges and obligations.

Introduction

The South Central African Region

The South Central African region comprises Malawi, Zambia and Zimbabwe. The region is bound by the latitudes $8°$ 25' and $22°$ 30′ South and longitudes $23°$ and $36°$ East. The region is wholly land-locked, the nearest points to the oceans being over 100 km to the Mozambique Channel and 900 km to the Atlantic Ocean. The total land area is about 1.25 million km². Climate is broadly divided into two distinct seasons - a summer rainfall season starting November and ending in April, and a prolonged dry winter season. This has a marked influence on the fruiting times of fungi and the timing of collection forays - which in our region are best productive in February and March. The mean annual rainfall is 635-1500 mm per annum. Mean temperatures are 18°-21° C in areas of a latitude range of 900 to 1200 m.a.s.l. (about 70% of the land area). Wild and Barbosa's (1967) *Vegetation Map of the Flora Zambesiaca Area* presents a floristic and physiognomic classification of the region, in addition to Mozambique and Botswana, the area draining the Zambezi River.

The characteristic vegetation type is that of the Leguminoseae genera *Brachystegia*, *Julbernardia* and *Isoberlina*. Such dry deciduous forests are termed miombo - they are widespread and predominant, covering up to half of the area of the region, at medium to high altitude and rainfall areas, on soils derived from

granite, schist, quartzite, gabbro or shale. The miombo ecozone - or miombo region - also extends into southern Zaire, northern Mozambique and parts of Tanzania and Kenya. Other forest types found in the region are the Zambezi Teak Forests, in which *Baikiaea plurijuga* is the dominant species (on the Kalahari Sands in western Zimbabwe and south-western Zambia, where rainfall is generally low). These forests also occur in parts of Angola and Botswana. There are small relic swamp and evergreen forests in high rainfall areas, containing the only coniferous species of the region (*Juniperus, Podocarpus* and *Widdringtonia*). Typical savanna woodlands are associated with some impediment in drainage and species of *Acacia, Combretum* and *Terminalia* predominate, though there are several secondary associates. In the lower elevation areas *Colophospermum mopane* predominates, especially on calcareous soils. Forested areas are interspersed with shallow depressions or drainage canals that are seasonally waterlogged (dambo or vlei), forming grasslands. Considerable development of plantations with exotic species (mainly *Acacia, Cupressus, Eucalyptus* and *Pinus*) currently totalling 2 500 km^2 has taken place within the last 4-5 decades

The assemblage of fungi in South Central Africa is large and heterogenous. It is impractical and probably unadvisable, considering the variety of species and the constellations of characters they posses, for one to sufficiently and efficiently address aspects of their taxonomy, ecology, distribution etc. - floristics - in this presentation. I will therefore, focus on the macrofungi, a group I have also studied for some years now.

Importance of fungi

The importance of and uses of fungi are diverse, some being detrimental to mankind, crops and animals. The presence and importance of fungi in agricultural and forestry systems as pathogens, saprotrophs and symbionts is well recognised. Their use in wine-making and beer-brewing industries, as food, medicines and mycopesticides, and in other novel biotechnological applications have been tapped to a variable extent in the region (Subramanian, 1992, Hawksworth, 1993, for the general importance of mycology to development). The applied mycology aspects attract the bulk of funding from public and private institutions. Generally, studies on fungal taxonomy and ecology, are regarded as being of fundamental importance only, and have received limited attention.

STATUS OF MYCOLOGY RESEARCH AND TAXONOMIC STUDIES

Meaningful mycology research is conducted by Universities, Departments of Agriculture and Forestry, some parastatals and a few private organisations.

Parasites

Comprehensive lists of fungi of pathological importance in agriculture (Rothwell 1982, Angus 1962-66, Doidge 1950) and forestry (Masuka and Ryvarden 1992a) have been compiled. Macrofungi on living forest trees (Table 1) have only been collected for serious study recently. The fungi play an important role in tree succession and hence stand and ecosystem dynamics. *Fomitopsis widdringtoniae* Masuka & Ryv. is commonly found on *Widdringtonia nodiflora* in Malawi (Masuka and Ryvarden, 1993a), and is associated with the widespread decline of the host species. *Armillaria, Amauroderma* and *Ganoderma* species are the most common

fungal causes of mortality in indigenous tree species. Heart-rot in indigenous merchantable timber species (e.g. *B. plurijuga* and *P. angolensis*) may reach an incidence of up to 19% in logs (Masuka and Ryvarden 1993b). This has prompted some recent studies (including those on the taxonomy and ecology of causal fungi) on the subject in Zimbabwe.

Table 1. Macrofungi associated with living trees in South Central Africa.

Species	Host	Parasitised part[2] (rot)
Abortiporus biennis (Bull.: Fr.)Sing	Bu,	root, butt
Amauroderma argenteofulvum (Van der Byl)Doidge	Bs, Jg	root
A. fuscoporia Wakef.	Bs, Jg	root
A. preusii (Henn.) Steyaert	Bs, Jg	root
Armillaria (Fr.) Kummer spp.	Bs, Jg, Pc	root
Aurificaria indica (Mass.) Reid	Csp	heart
Fomitopsis widdringtoniae Masuka & Ryv.	W	butt
Ganoderma australe (Fr.) Pat.	Kn, Bg	heart, butt
G. colossum (Fr.) Baker	Cm	root, butt
G. eminii (Henn.) Ryv.	Bs, Jg	root, butt
G. lucidum (Fr.) Karst.	Bs, Jg, Pc, F	root, butt
G. sculptrutum Lloyd	Bs, Jg (Eg)[*]	root, butt
Inonotus afromontanus Ryv. and Gilbn.	Psp	butt
Perenniporia martius (Berk.) Ryv.	Kn	heart
Laetiporus baudonii (Pat.) Ryv.	Bs, Jg	root
L. percisinus (Berk. & Curt.) Gilbn.	Bu	heart
Phellinus allardii (Bres.) Ryv.	Bsp, Cm	heart
P. fastuosus (Lev.) Ryv.	Cm	heart
P. lamaensis (Murr.) Ryv.	Bu, Kn	heart
P. merillii (Murr.) Ryv.	Kn	heart
P. rimosus (Berk.) Pil.	Psp, Pa, Ba, Bp, Bs	heart
P. robustus (Karst.) Bourd. & Galz.	Bu, Bs, Cp	heart
P. senex (Nees. & Mont.) Imaz.	Bs, Jg	heart
P. wahlbergii (Fr.) Reid	Bu, Bs	heart

[1] Bp, *Baikiaea plurijuga*; Bg, *Brachystegia glauscesens*; Bs, *Brachystegia spiciformis*; Ba, *Burkea africana*; Cm, *Colophospermum mopane*; Csp, *Combretum* sp.; F, *Ficus* spp.; Jg, *Julbernardia globiflora*; Kn, *Khaya nyasica*; Pa, *Pterocarpus angolensis*; Pc, *Parinari curatelifolia*; Psp, *Phillipia* sp., W, *Widdringtonia nodiflora*

.[2] But-rot defined as up to 1 m above ground; higher than that the term heart-rot applies.

[*] The fungus has recently adapted onto *Eucalyptus grandis* in Zimbabwe and causes up 4% annual mortality in high risk areas whose previous indigenous vegetation is *B. spiciformis* or *J. globiflora* (Masuka and Nyoka, 1994).

Mushrooms and mushroom poisoning
Mushrooms are highly rated on the local gastronomic scale, have a unique contribution to the diets of many people, and are extensively collected during the rainy season (December-March). Country and regional guides to mushroom identification and mushroom poisoning have been produced. Morris's (1987) *Mushrooms of Malawi* and Storrs and Piearce's (1982) *Do Not Eat These*, Piearce (1981a) and more recently Ryvarden, Piearce, Masuka's (1994) *An introduction to the Larger Fungi of South Central Africa* are useful starting points for anyone studying mushrooms in this part of the world. Throughout the region fungi are normally equated with mushrooms (Khonga 1994, Masuka 1992a, Morris 1984,

1987) and traditional classification systems - passed orally from one generation to the next - have existed to this day, though with urbanisation local, elderly people remain the only repository of such knowledge. The classification system is based principally on colour (there are 4-5 in the region), aroma, texture and taste. In Nigeria the shape, texture, taste and maturation period are the major taxonomic criteria employed by local people (Alabi 1994). A start has been made in Zimbabwe to prepare a checklist of vernacular names of fungi (in different languages and dialects) and to provide their English equivalents (where applicable) and scientific names. There is substantial folklore associated with edible and poisonous mushrooms though there are a few records of the use of macrofungi in traditional medicine, or for ritual purposes, and fungi are rarely used in artwork (Masuka 1992a, Piearce 1981a, Morris 1984, 1987). *Perenniporia mundula* (Wakef.) Ryv. is the only medicinal fungus used throughout the region, for treatment of pleurisy and impotence.

Termitomyces, *Cantharellus*, some species in the Russulales and Boletales are regarded as delicacies. A representative number of these, and other macrofungi, have been included in the book by Ryvarden *et al* (1994). *Amanita zambiana* Pegler & Piearce, confined to South Central Africa and southern Zaire, is abundant and widely collected for consumption. The hard-textured species such as *Lentinus cladopus* Lev. and *Polyporus tenuiculus* (Beauv.) Fr. are edible but seldom collected; for consumption during the off-season.

There are about seven common *Termitomyces* species among which four are highly regarded. They have been studied in greater detail in Zambia (Piearce 1987). There are approximately 1 800 termite species in Africa and South East Asia among which some 100 species in six genera grow, among them, about 20 species of *Termitomyces* (Bels and Pataragetvit 1982). The associated mounds can be invisible to about 10 m high. The relationship between *Termitomyces* and termites is an example of obligate symbiosis. The only other similar association of insects and fungi is that of the Attini, confined to Central and South America, which have been reported to grow a *Leucocoprinus* sp. (Holldobler and Wilson 1990). There is no evidence that the complementary habits of *Termitomyces* and *Leucocoprinus* sp. are mutually preemptive involving competitive exclusion, or accidental, reflecting the rarity of evolution along that path.

Amanita, Cantharellus, Lactarius and *Russula*, and also *Termitomyces* species cannot, as yet, be cultured. Their continued presence will depend on the protection of their natural environments. Mushroom collecting is regarded as a women's task. Mushroom productivity studies (counts by species and basidiocarps, basidiocarp diameters, determination of fresh and dry weights and nutritive values) in miombo forests in Zimbabwe have only been recently initiated. Aspects of mushroom collection, processing and consumption (and utilisation) patterns; importance in rural economy; and valuation, marketing and trade will also be considered. Such studies should result in a further understanding of their taxonomy and ecology.

Several cases of mushroom poisoning are reported each year, and children are most vulnerable. Most cases of poisoning reported generally fall within the class of gastrointestinal irritants (Ryvarden *et al.* 1994). Species (authentically) associated with poisoning are *Chlorophyllum molybdites* (Meyer: Fr.) Masse, and some *Amanita* species which are commonly mistaken for *Macrolepiota* and *Agaricus* species. Mushroom poisoning has attracted considerable attention from the print and electronic media in the region. Publications on the subject are by Flegg (1981),

Gelfand and Harris, (1982), Robertson (1983) and Sharp (1983) for Zimbabwe; Storrs and Piearce (1982) for Zambia, Morris (1987) for Malawi, and (Ryvarden *et. al.* 1994) for the region.

Following introduction of exotic plantation species (*Pinus* spp.) poisonous species of *Amanita* [*A. muscaria* (L.:Fr.) Hooker], but also edible species such as *Boletus edulis* Bull.:Fr. and *Suillus granulatus* (L.:Fr.) O. Kuntze have become naturalised in those plantations. Most locals regard macrofungi under exotic plantations with very deep suspicion.

Fungi on timber in service

Recent studies in South Central Africa have also focussed on the identification of decay fungi on timber in service, ascertaining their temperature and moisture characteristics in culture, determining frequency of occurrence and extent of damage they cause, and on aspects of their control (Masuka and Ryvarden 1992). There is even greater scope for such studies as the construction of timber houses is now permitted in Zimbabwe (Standards Association of Zimbabwe, 1990). Furthermore, fungi responsible for deterioration of paints and other products may also be studied. Some survey work on the fungi causing decay of mine timber has also been conducted. The studies have revealed the presence of *Gyrodontium boveanum* (Montagne) Maas G. as an important rotter of mine timber, *Coniophora puteana* (Fr.) Karst., *Antrodia vaillantii* (Fr.) Ryv. and *Gloeophyllum trabeum* (Fr.) Murr. in pine timber, and *Coriolopsis polyzona* (Pers.) Ryv. and *Trametes vesrsicolor* (Fr.) Pil. in hardwood timber (Masuka 1992a).

Saprotrophic and mycorrhizal macrofungi

Most fungi in the region are saprotrophs on dead wood and on leaf litter, being inconspicuous to the unaided eye to large and sometimes brightly coloured species. The only well studied group is the Aphyllophorales (Masuka 1992b, Masuka and Ryvarden 1992b, 1993c, Mswaka 1992). This is so because the fungi are easy to collect and store. Collections in the region have yielded much information, and currently the region is better explored mycologically than most parts of Africa. Studies on the agarics and boletes have recently been resuscitated following collaborative collection trips with institutions and individuals in the United States of America.

The taxonomy of tropical fungi is in a continuing state of flux including that of relatively well studied groups such as the polypores (Ryvarden, 1994). New species continue to be discovered following thorough collecting (Masuka and Ryvarden 1993a) and many more polypores will be described soon from our region (Mswaka, Masuka and Ryvarden, unpublished). Such new discoveries have added to the instability of generic and species delimitations, whose descriptions are often based, for historical reasons, on temperate species. Current work on novel *Dichomitus* species from the region have repercussions on the generic delimitations of *Grammothele*, *Grammothelopsis* and *Megasporoporia*.

Collections of Aphyllophorales have yielded very valuable information (Table 2) and recent comparisons indicate that there are only 2-5% brown-rot fungi in the Polyporaceae in the tropics compared to 28-30% in temperate countries (Ryvarden, 1994).

Table 2. Ecological roles of Aphyllophorales in Zimbabwe.

Family	Number of Species		
	Mycorrhizal	Parasitic	Saprotrophic
Corticiaceae	0	0	7
Hymenochaetaceae	0	11	28
Lachnocladiaceae	0	0	6
Coniophoraceae	0	0	8
Ganodermataceae	0	8	11
Hericiaceae	0	0	11
Polyporaceae	1	11	106
Punctulariaceae	0	0	1
Schizophylaceae	0	0	2
Steccherinaceae	0	0	2
Stereaceae		0	2 4
Thelepohoraceae	1	0	0

source: Masuka and Ryvarden (1993c)

The majority of the recorded species found in a study by Masuka and Ryvarden (1993c) had di- or tri-mitic systems of hyphae (Table 3), and there were significantly ($x^2 = 23.24$, P < 0.01) more monomitic species in higher rainfall areas (Afromontane and moist ever-green forests) than in the drier areas (Baikiaea and Mopane forests).

Within the various forest types the species occurring there tend to be predictable. Thus while *Phellinus rimosus* is a common species in mopane woodlands *Amauroderma* and *Ganoderma* species do not occur there but predominate in miombo woodlands.

Table 3. Hyphal system and vegetation type.

Forest Type	Number of Species		
	Monomitic		Di-/Tri-mitic
Afromontane	3	(38)	5
Baikiaea	3	(9)	29
Deciduous tree savanna	3	(10)	28
Eucalypts	7	(22)	25
Miombo	12	(15)	67
Moist evergreen	24	(26)	72
Mopane	3	(10)	26
Pine	22	(41)	32

The figures in parenthesis indicate the percentage of species with a monomitic hyphal system.

An analysis of the species of the Coniophoraceae (Table 4) shows that only a few have been found in Africa. The data was compiled from various sources including Ginns (1976, 1978, 1982), Gilbertson and Ryvarden (1987) and Masuka and Ryvarden (1991). These observations are based on the morphological species concept. It is not known whether some of the species found in temperate

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environments are present in the tropics in a vegetative state but fail to produce basidiocarps because of adverse climatic and other conditions. One inherent flaw in such comparative mycogeographical studies is the dilemma of distinguishing between non collection and non occurrence of a species in an area.

Table 4. Representation of genera of the Coniophoraceae in Africa.

Genus	Number of species in	
	Genus	Africa
Coniophora	15	8
Corneromyces	1	1
Gyrodontium	3	2
Jaapia	2	0
Leucogyrophana	9	1
Meruliporia	1	0
Pseudomerulius	1	0
Serpula	3	1

The Myxomycetes are extremely common but have not been collected for serious study in the region. Species of *Lycogala* are quite common in sheltered moist places. Among the common Ascomycetes known here are *Cookeinia* (Sarcosomataceae) though the whole group has been poorly studied. *Daldinia, Hypoxylon* and *Xylaria* species (Xylariaceae) are also quite common but the species have not been identified with certainty. Among the Heterobasidiomyectes only the common species of the Auriculariales, in *Auricularia*, and the Dacrymycetales, such as the conspicuous *Dacryopinax*, are known and often collected. The other order, the Tremellales, has not been studied in this part of the world.

Studies on the Agaricales of the region have been conducted by Pegler (1982), Pegler and Piearce (1980), Piearce (1981a, 1987), Morris (1987) and Ryvarden *et al* (1994). Pegler's (1977, 1986), *A Preliminary Agaric Flora of East Africa* and *Agaric Flora of Sri Lanka* have many applicable keys to agarics of South Central Africa and have formed a basis on which to build local knowledge. Studies on agarics have been most intensive in Zambia. However, a thorough and systematic inventory of the whole group has not been undertaken. Among the notable species are the poisonous *Chlorophyllum molybdites*, which can be mistaken for some *Agaricus* or *Macrolepiota* species. *Collybia aurea* (Beeli) Pegler (Tricholonmataceae) is a tropical African species whose dense and clustered habit makes it easily recognisable. The Russullales have also not been studied in any great detail. A few notable species in the genera *Lactarius* and *Russula* are however, confined to this region. *Lactarius kabansus* Pegler & Piearce is only known as yet from Zimbabwe and Zambia, in miombo woodlands. Perhaps the least studied group of macrofungi is the boletes which occur scattered throughout miombo woodlands. Among the poorly studied Aphyllophorales are resupinate species - the 'corticiums' including species in Hymenochaete (Hymenochaetaceae). The gasteromycetes too have been poorly documented, though some preliminary work was conducted in Zambia (Piearce 1981b). A notable species in the Lycoperdales (Broomeiaceae) is *Broomeia congregata* Berk. only known in Africa south of the Sahara, often close to *Acacia* tree species. Another distinctive fungus is *Podaxis pistillaris* (Linn.:Pers.) Fr.

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(Podaxales) a tropical and sub-tropical species, in Southern Africa found mostly on termite mounds.

Taxonomic approach

The biological species concept is the basis of taxonomy in fungi, though there has been much debate on this subject (Clemencon 1977, Parmasto 1985, Boidin 1986, Hallenberg 1988). Morphological and anatomical characters of the basidiocarp, type of rot, reaction with selected chemicals, host range and specialisation and distribution (mycogeography) are routinely used in taxonomy. The approach is simple, inexpensive and pragmatic under our conditions were a greater number of species are unknown. As the taxonomic species concept emphasises the importance of developmental criteria, this has necessitated the analysis of the basic characteristics of the individual. Cultural, biochemical, incompatibility and genomic studies, and phenetic and cladistic analyses have not been used for fungi of the region to any great extent. Such studies could solve some delimitation problems, and are especially useful in economically important species and genera (e.g. *Armillaria*). A broader consideration of taxonomic characters is often done in mycologically better known regions, where molecular studies have gained prominence; and phenetic and cladistic analyses yield most valuable information there. However, there is hardly need for competing taxonomic approaches - the classical and culture-based methods - rather they should be regarded as being complementary. Indeed, in several cases the modern methods have corroborated delimitations arrived at using the classical or "conventional" taxonomic methods.

Morphological characters are polymorphic and it is impossible to reveal convergence phenomena or parallel evolution from their analysis. *Funalia leonina* Kl. is primarily separated from *Trametes* by the presence of a thick mat of hairs on the upper surface of the basidiocarp. The presence of this feature in the species might just be an adaptation to predation by insects. Similarly, *Coriolopsis* species are separated from *Trametes* species by the coloured vegetative hyphae, and *Pycnoporus* is recognised as a distinct genus because of the presence of cinnabarin. *Flavodon flavus* (Kl.) Ryv. is separated from *Irpex* by its yellow colour, but is micromorphologically similar - also having simple-septate hyphae and encrusted cystidia. An in depth analysis of the species' cultural and biochemical characters might reveal their true affinities. Furthermore, the yellow colour often fades with age.

Only a few species are known in culture (11 500 strains out of an estimated 254 000 strains in herbaria around the world or 17% of known - and only 0.8% of estimated number of - species present in the world, see Hawksworth 1994a,b). The rapid disappearance of species due to deforestation and other causes has given priority to less tedious, but workable approaches to taxonomy, and to collection for ex-situ conservation purposes in general.

In recent collaborative studies on the genus *Armillaria* the limitations of the morphological species approach have been poignantly manifold. Various features (and methods) are now being used to differentiate the *Armillaria* species in culture, to complement classical methods (Table 5).

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Table 5. Methods used to group *Armillaria* species from tropical Africa.

1. Fruiting in culture
2. Observation of morphology of basidiocarps, conditions conducive to fruiting, frequency of fruiting, morphology of single-spore isolates
3. Pairing tests
4. Comparison of growth in culture at different temperatures
5. Comparison of subterranean rhizomorphs obtained in a mist case
6. Biochemical and molecular analysis of proteins: esterase, whole cell DNA by hybridisation of restriction digests with mtDNA and random amplified polymorphic DNA analysis
Source: Mohammed and Guillaumin (1994).

Basidiocarps of the fungus are rarely produced under our conditions (Masuka, 1994). Studies by the "Zimbabwe *Armillaria G*roup", under the EEC Project on African
Armillaria coordinated by the Oxford Forestry Institute, focussed on growth features and biochemical characteristics of the fungus in culture (Mwenje and Ride 1994) and on species screening for resistance and nursery pathogenicity experiments.

CONSTRAINTS

Priorities and funding
There is a lack of adequate, assured and continuous funding, as taxonomic studies have been relegated from the priority list; and applied pathology/mycology, including biotechnology, have gained prominence. It is however, important to note that knowledge of biodiversity, the .raw material for biotechnologies for agricultural development' (Boussingeut 1991), (which is heavily dependent on taxonomic studies) is (still) poor and fragmentary. Among the reasons for the lack of taxonomic studies is perhaps the opinion that taxonomic studies have no commercial value or a place in applied research. The importance of these studies has been underplayed leading to a marginalisation of the subject, hence taxonomy has become peripheral to the fields of agricultural or forest pathology. There is competition with other fields for funding, in addition to status and credibility. Only three out of 18 papers presented at the First African Regional Mycology Conference in Mauritius in 1990 dealt with fungal taxonomy (Hennebert 1994).

Countries in the region are pursuing structural adjustment programmes. These have generally, led to a reduction in public expenditure. In any budget cuts research operations, by their nature, usually suffer first and most. The salaries budget for some research institutions already account for 70% of the operational budget, further reducing financial resources allocated to research work.

Personnel
There is a lack of qualified staff. Those that have training in taxonomy often conduct taxonomic studies on a part-time basis, as there is no full-time taxonomist in the region. There is also a lack of status, recognition and generally, inadequate career paths for researchers in developing countries, and taxonomists are no exception. At this symposium whose theme is "Tropical Mycology: quo vadis?" only two out of the seven speakers are from the tropics. The respective number of permanent scientists listed in the directory of African mycologists for Malawi, Zambia and

Zimbabwe are only 1, 3 and 7 (Buyck and Hennebert 1994). Only Zimbabwe has the personnel capable of conducting systematic inventories of macrofungi.

Literature

There are no monographs for the region. One has to depend on literature from elsewhere. The shortage of relevant information on tropical species has made any pioneering study a frustrating experience. With regards to the polypores Ryvarden and Johannson's (1980) *Preliminary Polypore Flora of East Africa* provides a valuable starting point, so do Pegler's (1978, 1986) monographs of agarics.

Facilities

The facilities available do not allow for advanced methods to be employed, so studies employing molecular approaches to taxonomy (enzyme, DNA analysis) can only be conducted at Universities (there is a shortage of equipment there as well), and will probably remain being of an academic interest only for the foreseeable future.

In all three countries the macrofungi herbaria fall under different institutions: the Zambian collection is at the Forestry Research Headquarters (*ca.* 2000 specimens), in Malawi the collections (*ca.* 500 specimens) are at the National Herbarium and Botanic Garden, while those in Zimbabwe are at the University of Zimbabwe (on temporary transfer to the Forestry Commission, Forest Research Centre since 1989, *ca.* 3000 specimens).

Methodology

Our priority in the region is to collect and document species before they disappear. This calls for the morphological and anatomical species approach to taxonomy. In well studied regions, studies have now concentrated on elucidating relationships between taxa and solving delimitation problems. Sometimes our approach (morphological) to species delimitation appears parallel to the biological species concept, and problems have been encountered in conclusively identifying species described on the basis of the latter concept. The shortage of equipment to conduct the required further analyses compounds the problem.

Communication and networking

Because of the constraints imposed by a shortage of funding scientists in the region do not travel often enough to meet and share ideas with peers. This has often led to unnecessary duplication, thus retarding the pace of progress in some studies. Joint project proposals are rarely made with institutions in the region. Opportunities for networking have often come from exogenous factors, being spear-headed from institutions outside the region.

WINDOWS OF OPPORTUNITY

The rate of deforestation on a global scale is estimated at 15 million hectares a year (UNEP, 1987) with greater losses taking place in developing countries and at present rates of deforestation about 40% of remaining forest cover might be lost by the year 2000 (Organisation for Economic Cooperation and Development, 1985). The estimated rate of deforestation is over 250 000 hectares annually in the region (Table 6). The causes are shifting cultivation, expansion of agriculture, cattle

ranching, the growth of industrial mining, and timber and wood exploitation and rapid urbanisation. These are exacerbated by high population growth rates of up to 3% per annum.

During the past ten years the need to preserve (plant) diversity has been popularised culminating in the conniving of the Earth Summit (and Agenda 21) in Brazil in 1992. Funding for conservation efforts has been mobilised and much of this has targeted developing countries. However, the need for a holistic ecosystem management approach has also illuminated the need to incorporate other components.

Table 6. Deforestation rates in the region.

Country	Total Area (x1000 km^2)	Rate of Deforestation (% per annum)
Malawi	9408	0.93
Zambia	74072	0.53
Zimbabwe	38667	0.45

Source: Singh (1991)

Fungi are important components of forest ecosystems, recycling immobilised nutrients and, through symbiotic associations, directly contributing to the well-being of forests. However, at present rates of deforestation some species might even be lost before they are known. A window of opportunity has been opened for conserving and inventorying fungi, and to further study their ecology and biology. Indeed, more than ever before, there is now scope for assessing absolute, generic, ecological and mycogeographical diversity of fungi.

There is still a receptive audience (in both public and private institutions) willing to cooperate, collaborate and fund joint studies, and to assist in training developing-country scientists. The opportunity must not be missed.

There are vast opportunities for studying the mycorrhizal species in miombo woodlands, for their contribution to plant growth, and for opportunities to create manufacturing and processing industries to satisfy local and international demand for mushrooms and mushroom products. These studies will gain prominence due to the current wave of interest in non-timber forest products arising from a new realisation that woodland management is an integral component of social forestry programmes in the region.

A variety of national and regional literature has been published on mushroom poisoning because the only sure way to know whether a mushroom is safe to eat is to be able to identify it in advance accurately and with certainty (from own and others experience and books). Mushroom poisoning will continue to be an important public health concern. This should result in more taxonomic and ecological studies on mushrooms and related fungi in the region.

The biotechnological applications of fungi will soon feature prominently in the region, in concert with developments in other parts of the world. There is a search for emtomopathogenic fungi for use against scale insects (*Aspidoproctus* sp.) which have decimated extensive areas of *Brachystegia*-dominated forests in Zimbabwe. Such studies will, undoubtedly, have taxonomic and ecological aspects - which should contribute to a better inventory of microfungi of the region. Some work might

focus on elucidating the biochemistry of the curative properties of fungi such as *Perenniporia mundula*, for the academic and practical challenges it presents. The increasing use of genetically uniform crops and trees in monocultures could give rise to more disease problems. That should keep mycofloristic studies of this important group of fungi alive. In addition, biological control options in agriculture and forestry will gain prominence in line with the need to employ environmentally benign methods of pests and disease control. Finally, the search for secondary metabolites for use in pharmaceutical and other industries should spur mycofloristic studies in the region, as hitherto unknown or little explored habitats are thoroughly inventoried. Indeed, the diversity of tropical fungi has been recognised together with their potential use in biotechnology (Subramanian 1992), so an increase in their inventorying and methodic study is envisaged.

Herbaria must be regarded as assets, and studies in these could provide a basis for checklists, and indicate priority areas for further field collecting and documentation studies.

STRATEGIES

Training

There is need to build capacity for fungal inventorying, taxonomic and other studies. This can be accomplished by organising fungal identification training courses. The International Mycological Association (IMA), International Mycological Institute (IMI) and the Committee for the Development of Mycology in Africa (CODMA) are planning to organise a Regional Fungal Identification Training Course for Africans actively engaged in research in the fields of pathology and mycology during the early part of 1995. Short courses and exchange visits foster the sharing of information sharing, so should be encouraged.

Fungal inventorying

Intensified collection and documentation studies should be accorded the highest priority, otherwise many species will disappear before they are collected. Little explored habitats must also be inventoried. A series of ecological studies are either in progress or shall be initiated soon.

Literature

Access to literature should be improved through increased subscription to journals. Severe foreign exchange problems and the high subscription rates militates against these efforts -ultimately only a handful of journals might be subscribed to and, even fewer may be regularly available. Electronic literature databases such as CD-ROM, Internet etc. should be provided to allow for rapid and accurate literature searches. However, to enable this funding will need to be sought.

Mobilise, organise and educate

There is need to sensitise and organise schools into amateur clubs; organise poster competitions in schools; create awareness and fungal conservation societies; involve the media (regarding mushrooms); and publicise fungi through stamp and other anniversary commemorations. The importance of fungi in the maintenance of ecosystem diversity through symbiosis and saprotrophic activities, in biotechnology, as food and medicines; and for aesthetic purposes, should be continually highlighted.

Environmental impact assessment studies should have a fungal component. Mycologists should mobilise and organise themselves and others to lobby governments, public and private organisations for this component to be included wherever such studies are being conducted. The importance of fungi as bio-indicators of change (habitat disturbance, pollution etc.), if clearly articulated, should result in an increase in fungus-related environmental studies.

Herbaria

Herbaria should be publicised for their scientific, educational, conservation and other purposes. This might lead to more collections being undertaken by individuals and local societies. Presently herbaria are grossly underutilised. Herbaria are a repository of myco-diversity - analogous to seed centres and gene banks - and must be regarded and treated as assets.

Documentation

Mycologists in the region will need to devote a considerable proportion of their time to documentation studies. A checklist of macrofungi recorded in Zimbabwe is now being prepared. There are no pathology or mycology newsletters, nor journals in the region. The launching of the *African Mycology and Biotechnology Journal* in Cairo, Egypt, in 1992 should provide an outlet for relevant publications (and African perspectives). Exploratory work on starting a regional newsletter has just commenced.

Cooperation, collaboration and networkin

Cooperation, collaboration and networking (with individuals and institutions within and outside the region) are prerequisites for any meaningful progress to be achieved on mycofloristic studies of the region. Attendance at conferences, symposia, workshops and seminars should further provide important fora for the exchange of ideas and information. Meagre financial, human and physical resources can then be appropriately deployed resulting in a sharpening of research focus and rapid progress. Faster methods of communication are needed. International collaborative/cooperative fungal inventorying efforts should be initiated, but need to be clearly defined and well planned (in the wake of property rights) and, of necessity, should encompass and build up and equip national centres in the respective countries.

CONCLUSION

The success of strategies outlined above depends on the availability of funds; local, regional and international support; and perhaps most importantly, the whims and aspirations of scientists in the region. We must initiate intensive studies soon, and even if we do not achieve our goals, in the words of Robert Louis Stevenson, '...to travel hopefully is a better thing than to arrive, and the true success is to labour.'

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STRATEGIES FOR THE DEVELOPMENT OF TROPICAL MYCOLOGY

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Introduction

The need to develop biosystematics generally and mycology specifically has been the subject of some pertinent interventions during the last five to six years (Hawksworth and Bisby 1988, Hawksworth 1991, Hawksworth 1993, Peerally 1994, Haskell 1994, Rossman 1994, Zedan 1994, Bennett 1994, Jones 1994). Earlier Subramanian (1982, 1985) had highlighted various manners by which mycology could promote development in the tropics. Subramanian (1986) observed that there was no organisation in South or South-East Asia which could help anyone in the identification of fungi important in agriculture, forestry, medicine, public health and industry. We could very much make a similar observation today as we approach 2000A.D. It must be said that the development of mycology is of relevance not only to the tropical countries but to all industrialized countries as well. Any strategies for mycological development inescapably have to take into account the synergistic interaction between developing and developed countries.

This paper will attempt to review briefly the relevance of mycology to development, the various efforts in the tropics and in the North aimed at promoting biosystematics and/or mycology. These interventions and efforts already incorporate recommendations for the development of mycology world-wide and what is now needed really is to seek funds to support mycology in a more integrated and organised manner.

Relevance of Mycology to Development

Hawksworth (1993) detailed out, prioritized and categorized the relevance of mycology to economic development with particular reference to the tropics and a summary of his ideas is as follows:

(a) Food Security
      Diseases in the fields
      Post-harvest losses
      Disease diagnosis
      Biocontrol
      Quarantine
      Chemical control
      Plant breeding for disease resistance
      Mycorrhizas
      Edible fungi
      Mycoprotein
      Mycotoxicology
      Fermented foods and beverages

(b) Waste Utilization
    Upgrading of wastes into animal feeds
    Composting
    Biodegradation for paper making
    Recycling of organic wastes
    Bioremediation
    Limitation of organic pollutants

(c) Public Health
    Food safety
    Mycotoxins
    Mycoses
    Fungi in AIDS
    Fungi in allergy

(d) Biotechnology
    Fungi as an unevaluated resource
    Pharmaceuticals
    Industrial enzymes
    Pesticidal properties
    Fermentation technologies
    Flavours, fragrances
    Delignification

(e) Maintenance of Biodiversity
Fungi play an important role in the maintenance of biodiversity through the maintenance of ecosystem function. Thus fungi are useful to higher plants in mycorrhizas, fungi and fungal enzymes play a key role in the digestive tracts of many feeding insects and even in the stomach of ruminant mammals. Fungi are important in nutrient cycling and natural biodegradation. Other fungi and various lichens have roles as bioindicators of ecological continuity.

Status of Mycology in Tropical Countries

While a few tropical countries like Australia and South-Africa have attained at least the critical mass in terms of applying mycology to development, most other tropical countries significantly lag behind. Hawksworth in his keynote address at the Second African Regional Mycology Conference (Hawksworth 1993) said: "Mycology has an immense amount to contribute to key aspects of development, but the subject is rarely accorded the attention it merits in national programmes aimed at increased sustainability. In the Tropics, and less developed regions generally, this is particularly true. Yet it is in those very areas that mycologists are generally few in number, and have scant resources?"

All aspects of mycology need strengthening in tropical countries, especially in view of the various continua such as mycology-biotechnology, mycology-agriculture, mycology-human health, mycology-industry. Areas which need urgent attention are crop diseases, post-harvest losses, new biological control possibilities, mycorrhizas, food storage and mycotoxins, edible fungi, the microbiology of fermented foods and beverages, waste utilization, environmental bioremediation,

fungi causing human diseases and the isolation of novel and desired strains. (Hawkswort 1993).

In terms of constraints the major problems are the scant human resources, information and reference collection resources and funding for research, training and development work in mycology, as well as the inadequacy of appropriate career structures in Universities and research institutions, government bodies and in the private sector.

The International Mycological Directory (Hall and Hawksworth, 1990) lists 96 mycological organisations and institutions in the North, compared to 23 in the South of which 11 are in Australia alone. Only Argentina, Australia, India, Mexico, and the Republic of China have mycological societies. No African country has its own mycological society. Most lack even basic check lists, only nine of 53 African countries have any mycological collections included in the latest edition of Index Herbariorum (Holmgren *et al.* 1990). Only five African countries have culture collections listed in the current World Directory of Culture Collections (Takishima *et al.* 1989). Checklists are virtually non-existent.

Hawksworth (1993) went on to say that mycologists in Africa are isolated, and spatially remote from other mycologists, the major work in African mycology continues to be authored by Europeans.

Although a few mycologists in Asia, Latin America and Africa have lived up to international standards in their contribution, the magnitude of mycological problems in the tropics remains totally out of proportion with the available resources. The most poorly equipped are Africa, Central America and the Caribbean.

The IMA Regional Committees for Africa, Asia and Latin America are attempting to popularize mycology in their respective regions, but the meagre financial resources at their disposal barely allow meaningful activities to be organised, except for the occasional regional conferences. Plans of action which have been worked out as part of the deliberations of regional meetings have not taken off in most cases due to problems of networking resulting from financial constraints.

International and National Organisations/Institutions Promoting Mycology

Associations and Societies

In spite of the strong linkage in the continuum mycology-biotechnology, mycology is a discipline which cannot muster the media coverage not the public or private funding as biotechnology. There are good signs however that mycology, which has a brighter future now than was felt say a decade ago, is becoming more organised and coordinated. The International Mycological Association and D. L. Hawksworth have contributed significantly to the popularisation of mycology. The International Mycological Congress, formerly held every 6 years or so, is now organised every four years. IMA Regional Committees in Europe, Asia, Latin America and Africa are actively having their own regional conferences and some other activities. Other subcommittees of the IMA have contributed significantly in rationalizing nomenclatural problems.

The International Society for Human and Animal Mycology (ISHAM) formed in 1954 encourages the practice and study of all aspects of medical and veterinary mycology, and had 842 members from 68 countries in 1990. It publishes the Journal of Medical and Veterinary Mycology. It holds regular international meetings and

occasionally ad hoc meetings and workshops.

The International Society of Mushroom Science (ISMS) focuses on fungal biotechnology, edible fungi, macromycetes and holds regular international congresses, and symposia on specific topics.

Other international mycology organisations are the Mycology Division, International Union of Microbiological Societies with interests in biotechnology, medical and veterinary fungi, micromycetes, mycotoxins and yeasts; the International Association of Lichenology and the International Pythium Group. The recently established World Society of Mushroom Biology and Mushroom Products (WSMBMP) is interested in all aspects of edible and non-edible, poisonous macrocycetes including those having medicinal attributes.

Institutes

International Mycological Institute (IMI)

A major world centre of excellence for the systematics of filamentous fungi, it provides specialist support and associated services in all aspects of applied mycology, including information and training.

Centraalbureau voor Schimmelcultures (CBS)

An institute of the Netherlands Academy of Sciences, it deals with all aspects of mycology, provides information and identification services and organises training courses regularly.

Mycotheque Université Catholique de Louvain (MUCL)

MUCL is part of the Belgian Coordinated Collection of Microorganisms (BCCM) financed and coordinated by the Science Policy Office of Belgium. Besides being a reputable fungal reference centre, MUCL encompasses systematic and applied mycology. It also offers mycological services and customized training facilities.

American Type Culture Collection (ATCC)

Its primary aim is to acquire ex-type cultures of all named taxa and strains of scientific and commercial interests. It provides information and identification services. It organises training courses and workshops regularly.

US National Fungus Collection (USDA)

It is dedicated to the systematic study of plant pathogenic fungi, those useful in biological control and of plant quarantine significance. It has developed an important computerized information system for the identification of pathogenic fungi.

Microbiological Resources Centres (MIRCEN)

The Microbiological Resources Centres consist of a network of centres of excellence in both tropical and developed countries dedicated to the application of microbiology to development, and emphasize bacteria as well as fungi. It is one of the organisations dealing with mycology that are mainly funded through the UN system. MIRCEN has also contributed significantly to training of personnel in applied microbiology.

The Biodiversity Debate

The realization that biological sciences, molecular biology and biotechnology in particular, might hold the key to economic explosion and sustainable development in the decades to come is, as we know, significantly behind the global debate about biodiversity. The risk of environmental degradation with consequential loss of this biodiversity has also been an important element.

According to Hawksworth (1993) as little as 5% of the world's fungi have yet been described out of a mycobiota of some 1.5 million, of which the major part is found in the tropics. This mycobiota is a largely untapped resource in terms of biotechnology. Their exploitation for commercial applications has naturally raised the important question of intellectual property and patent rights. The 1992 Biodiversity Convention of Rio, signed by 153 countries, clearly establishes:

(a) the sovereignty of Governments over their biodiversity,
(b) the principle of poor countries having a claim on the international community to help meet some of the costs of conserving biodiversity for global benefit through such mechanism as the Global Environment Facility (GEF), and
(c) the need for mutually sharing the benefits accruing from intellectual property and genetic resources.

Hawksworth (1993) pointed out that the benefits from fungi and other biotic materials exploited in the Northern Hemisphere largely accrue to the companies found in the North. There is therefore a need for most tropical countries to themselves become involved in the isolation of novel and desired strains, and to regulate their supply to overseas companies under mutually agreed terms of payment and royalty (Hawksworth 1993)

Some Recent Initiatives for the Promotion of Systematics and/or Mycology

(1) The setting up of Regional Committees for the Development of Mycology by the International Mycological Association provides new opportunities to create greater awareness at the level of policy makers, industrialists, the scientific community and even in the public at large.
(2) The meeting organized in 1987 at the Royal Society by the Systematics Association on the occasion of its Golden Jubilee spearheaded a new vision about systematics. The opening paper (Hawksworth and Bisby 1988) entitled "Systematics: The Keystone of Biology" provided substantial proof of the indispensability of systematics to biology.
(3) The Convention of Biological Diversity and the emergence of the concept of sustainable development have had an important impact through the creation of a world realization on the need to study and conserve biodiversity.
(4) A study conducted by CABI at the request of ODA on the priorities of Biosystematics Research in Support of Biodiversity in Developing Countries with particular emphasis on microorganisms and invertebrates was completed in 1993 (Hawksworth and Ritchie 1993). The report made some very useful recommendations for the development of tropical systematics.
(5) The WEFSA (Workshop on the Ecological Foundation of Sustainable Agriculture) has been another critical thrust. WEFSA I (Hawksworth 1991) drew attention to the inadequate current capabilities in matters of systematics of

microorganisms and WEFSA III (Hawksworth 1994) examined this issue in depth and suggested ways to alleviate them.

(6) The Darwin Initiative for the Survival of Species, announced by John Major in Rio, focuses attention on strategies for the monitoring of biodiversity and on conservation and sustainable development. The Darwin Fellowship Scheme which includes awards mycology, is already operational.

(7) BIONET - International established by CABI at its 1993 Biennial Review Conference is a network with a global dimension and currently deals with arthropods, nematodes and microorganisms. BIONET is composed of subregional networks or LOOPS (Jones, 1994). The LOOPS will pool together resources to facilitate the pursuit of biosystematics endeavour using local resources. The LOOPS will coordinate and obtain support from the Bionet Technical Secretariat and from the world's major consortium of biosystematics institutions (BIOCON).

UNDP has already agreed to provide catalytic funding to BIONET International. The first BIONET LOOP was established in December 1993 for the Caribbean subregion (CARINET) involving 12 countries of the region. Similar LOOPS are in the pipeline for India, Eastern and Southern Africa. BIONET has an International Consultative Group with Dr. M. S. Swaminathan as its Chairman. CABI was asked by its 33 member countries at the Review Conference (14-17 June 1993) to do its utmost to assist the implementation of BIONET International.

(8) The U.S. National fungus collection has compiled electronic data from about 500,000 fungal specimens including rusts, smuts, polypores, asexual fungi and now ascomycetes as well. This database is important in providing identification of agriculturally important fungi and is not only useful for primary identification services, but also for substantiating new records (Rossman 1994).

(9) In terms of biochemical and molecular biosystematics, molecular studies are beginning to reveal hidden taxonomic criteria, especially where confusion exists with complexes of sibling species where molecular analysis may be usefully applied as with the former Armillaria mellea complex. (Rossman 1994).

Reckoning with Some Constraints

In the current attempt to organize, rationalize and consolidate ourselves as a world mycological community with common practices and objectives, we have to engage in some kind of corporate planning and in so doing we cannot escape analysing both strengths and weaknesses.

The following quotations speak for themselves:

1. "The practices of taxonomy and identification services are perceived as wrapped in white coats, obscured by Latin words, clouded in mists of camphor and formaldehyde, or even pickled in alcohol. They are seen as the preserve of an elite, to some extent parasitic, epiphytic, or at best, saprophytic upon the resources of other activities. Can this image be changed?" (Bennett 1994)

2. "Taxonomy must become an extrovert activity and escape the isolation of a Petri dish of introversion. The full value of taxonomic studies can only be demonstrated as a vital link in a process that creates income or achieves wider objectives". (Bennett, 1994)

3. "As a world community we have not come close to satisfy the most basic needs for identification services and resources to agriculture". (Rossman 1994)

4. "The identification and characterisation of important organisms depends directly on the state of systematic knowledge of those organisms. At present, for many groups of pest organisms, knowledge on which to base accurate identification and characterisation and an understanding of the relationships of target organisms to related species is lacking". (Rossman 1994)

5. "For both fungi and insects any attempt to identify agriculturally important organisms can be unsatisfying in that an accurate identification is difficult. Between 25% and 50% of the specimens submitted to the USDA systematic laboratories cannot be accurately identified to the species level". (Rossman 1994)

6. "Many of the organisms belong to large genera which have never been monographed so that identification to the species level is impossible". (Rossman 1994)

The Strategies

Hawksworth (1993) has clearly emphasized that prospects for developing mycology in the tropics are good provided the prerequisite organisation and planning can be effected by 1995. These prospects arise mainly from the new opportunities available through current awareness and interest in biodiversity and sustainable development, and through the Biodiversity Action Plans as proposed under the aegis of the Global Environment Facility.

It is evident from the points raised so far that the mycological scene globally is fairly buoyant with prospects of a better future in the years to come. It has been repeatedly emphasized how mycology hooks up with sustainable development, agriculture, industry, biotechnology, human health, food security, maintenance of biodiversity. Mycology is not and should not be sold as an applied science only. Fundamental mycology is an important as applied mycology. Mycology has both its Petri dish introversion and its biotechnological extroversion.

Another point should be emphasized. In the international, global outlook of mycology, in contrast to areas like the materials and the electronic technologies and others which are mainly the affairs of industrialized nations, an industrially useful fungal strain, for example, can be found anywhere but we need to know its identity and unravel its practical use.

The consolidation of mycology is naturally a priority for the tropical regions, but strategies for the development of mycology in the tropics should not ignore the fact that expanding facilities, research, infrastructure and services in the North will be of enormous benefits to the tropics.

The main actors in strategies for mycological development are:

(1)   The international societies and associations like the IMA, ISMS, IUBS, WSMBMP and their regional committees, if any.

(2)   The centres of excellence like IMI, ATCC, CBS, MUCL, USDA.

(3)   Networks like BIONET, MIRCEN.

(4)   Mycological laboratories in the North and South.

(5)   National, regional and international funding agencies.

Strategies for promoting tropical mycology will have as main ingredients:

(a)   Improving expertise of existing human resources through appropriate training opportunities.

(b)   Devising a Tropical Post Graduate Training Programme in Pure and Applied Mycology.

(c)　　Creating critical masses through national and regional pooling together of resources in priority areas.

(d)　　Creating national and regional mycological societies.

(e)　　Funding existing international mycological associations to enable them and their regional committees to operate efficiently.

(f)　　Encouraging the development of tropical centres of fungal identification through North-South joint ventures. Hawksworth (1992) emphasized that both national and regional capabilities in identification infrastructure are essential to meet the needs of agricultural and environmental services.

(g)　　Establishing an international task force (call it Project Neo-Saccardo) for the urgent preparation of monographs on important groups of fungi and available in electronic form. This would be of very significant help to taxonomists and non-taxonomists world-wide. In fact Rossman (1994) made the extremely pertinent observation that, in order to ease the identification requirements of both small and large scale countries, we need: (a) a world-wide network of primary contacts well trained and with relevant information tools at hand, (b) a cadre of back-up experts who provide identification of unusual species, and conduct research in systematic and (c) a mechanism to upgrade, revise and modernize the identification tools provided to primary contacts.

(h)　　Assisting developing countries to set up culture collections at least on a regional basis.

(i)　　Encouraging the development of centres of excellence in mycology in the tropics.

(j)　　Harnessing national and international support for established networks like BIONET and MIRCEN.

(k)　　For the purpose of attracting better funding possibilities it might be desirable to organize workshops/training seminars on topics which extrovert mycology: e.g. Mycology and Human Health (WHO might fund), Mycology and Pollutant Bioremediation (UNEP would be interested), Mycology and Industrial Development (UNIDO might certainly be interested), Mycology and Biotechnology, Mycology and the Maintenance of Biodiversity, Mycology and AIDS, Mycology and Allergy, Mycology and Protein Production, Mycology and Animal Feeds, Mycology and Fermented Foods, and many others.

The collective wisdom of the world's leading mycologists has been prominently seen at work during the past ten years or so, and it has started to yield positive results in terms of reasonable success in mobilising the scientific community, policy makers, and international agencies. The MUCL Centenary Celebration which focuses so much attention on tropical mycology is ample proof of the determination of the world's mycology community to function for the common good of humanity.

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THE ROLE OF THE MICROBIOLOGICAL RESOURCES CENTRES (MIRCENs) IN THE DEVELOPMENT OF MYCOLOGY IN THE TROPICS[2]

E.J. DA SILVA

Introduction

It is a pleasure to have the opportunity, albeit even in abstentia, to be associated with the Centenary Celebration of the Mycothþque de l'UniversitÚ Catholique de Louvain. In this regards, UNESCO notes with satisfaction the herculean work carried out over several decades under the inspiring leadership of Professor G; Hennebert in building up this culture collection of fungi. While in the 1950's the collection maintained a few hundred cultures of fungi, reference to the classical *World Directory of Collections of Microorganisms*, produced in 1972 by John Wiley & Sons, Inc., with the financial support of UNESCO, shows that MUCL then held 1183 species of fungi. Those were represented at the time by 14,500 cultures of fungi and 500 cultures of yeasts. Moreover, the interests of the collection were distinctly confined to agricultural and industrial mycology. Charging no fee for distributing and exchange of cultures, providing research qnd teaching services, MUCL had no published catelogue.

Twenty-two years later, the picture is different. From a one-time staff component of 1, the total strength has grown to 9. Moreover, a fee is levied for the distribution and exchange of 24,000 cultures of fungi, including 2000 cultures of yeasts. Storage, identification, training and consultation services are provided in the management of culture collection, propagation, culture and preservation methods, and in quaratine and shipment regulations. Still again, the interests of the culture collection have grown. These range from agriculture, applied and industrial microbiology, fermentation, freeze drying, food science, forest microbiology, and biodeterioiation, to systematics and taxonomy.Finally, in addition to sponsorship by the University, there has been support from the Belgian Science Policy Office, and the then Commission of the European Communities (CEC).

This admirable yet painstaking evolution of MUCL, in just two decades, from its early humble existence into its present day acknowledged national and international stature and status, is but a powerful reminder of the efforts and time required for the development of such culture collections in the developing word. Furthermore, the obstacles and challenges, encountered by mycologists in different regions of the developing world, are virtually the same as those encountered by virologists, protozoologists, phycologists and even bacteriologists.

Although well established fungal culture collections do exist, e.g. the Centraalbureau voor Schimmelcultures in The Netherlands, the CAB International Mycological Institute in the U.K., and the Mycological Culture Collection in the USA, proposals for funding and setting up such collections have often received

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token support. The stark reality of the scarcity of such resources in times of economic repression and emerging competitive priorities such as environmental management or greening of the deserts is to be found every time a current culture collection changes status to that of being an endangered culture collection -- be it mycological, viral, protozoal or bacterial in content.

This is evident from the excellent initiative of the World Federation of Culture Collections (WFCC) in releasing "Living Resources for Biotechnology" which is a series of practical books that provide primary data and guides to sources for information on matters relating to the location and use of animal cells, bacteria, filamentous fungi and yeasts. It is worth noting that each volune contains specialized information together with material on general matters (information centres, patents, consumer services, and international coordination of culture collection activities) that is common to each group treated, i.e. animal cells, filamentous fungi, bacteria and yeasts. Moreover, each volume also provides valuable information on resource centres located world-wide.

Of late much concern has been voiced about the lack of attention given to these research centres, and by consequence to specialized areas such as mycology, protozoology, entomology, and the like. This concern has been eloquently articulated in the run-up to and the follow-up of the 1992 U.N. Conference on Environment and Development in the Rio de Janerio, Brazil (Hawskwoth and Ritchie, 1993).

In order to address the topic of the assignment, a brief review of the MIRCEN network appears to be useful.

The international support of culture collections -- the treasure houses of the planet's microbial (and, of course, mycological) genetic heritage, can be traced back to the early catalytic support provided by UNESCO, in 1946. Several of today's well-known culture collections have been beneficiaries of such support (Da Silva *et al.* 1977). Moreover, support was extended also to relevant publication such as the *Internatinal Bulletin of Bacteriological Nomenclature and Taxonomy.*

In 1962, UNESCO's Twelfth General Conference adopted a resolution by the Government of Japan to initiate and intensify research and training activities in microbial biotechnology in view of the growing domestication and use, at that time, of microbial resources in the sector of food, energy, industry, medicine, and agriculture. As a consequence, UNESCO, in 1963, initiated a series of GIAM conferences and in subsequent years, undertook joint collaborative activities with the Panel on Microbiology of the International Cell research Organizations (ICRO), the International Association for Microbiological Societies (now known as IUMS), the International Organisation for Biotechnoloy and Bioengeneering (IOBB), and the World Federation of Culture Collections (WFCC).

The 16th. Session of UNESCO's deliberations in 1970 were enriched by a resolution from the Governments of Denmark, Finland, Norway and Iceland calling for the establishment of specialized microbial research centres in developing countries. This foresight by these Governments, in its historic perspective, was the essence of what today is known as the MIRCEN network.

The origins of the MIRCEN network can be traced back to the early days of the UNESCO/IRCO Panel on Mircobiology. Established in 1965, the Panel was principally concerned with:

(i)   the establishment of an international network for the preservation and exchange of cultures;

(ii)   the promotion of the use of micro-organisms as a natural resource; and,
(iii)   the world-wide training of microbiologists (Porter 1974, Bull and Da Silva 1983).

Following the United Nations Conference on the Human Environment at Stocklohm, 1972, experts from UNEP, UNESCO, and the international scientific community, represented in ICRO, met in 1974 and 1975 at the UNEP Secretariat at Nairobi, to formulate jointly a world-wide programme aimed at the presevation of microbial gene pools and to make them accessible to developing countries through the establishment of a network of microbiological resources centres (MIRCENs). Subsequently, a UNEP/UNESCO/ICRO project titled "Development of an Integrated Programme in the Use and Preservation of Microbial Strains for Deployment in Environmental Management" (July 1975 - October 1984) was formulated and submitted by UNESCO/ICRO, and approved by UNEP for implementation. The objectives of the MIRCEN network are:
 (i)   to provide a global intrastructure which would incorporate national, regional, and inter-regional co-operating laboratories geared to the management, distribution, and utilization of the microbial gene pools;
(ii)   to reinforce the conversation of micro-organisms, with emphasis on Rhizobium gene pools in developing countries with an agrarian base;
(iii)   to foster the development of novel technologies native to specific regions;
(iv)   to promote the economic and environmental applications of microbiology; and,
 (v)   to serve as focal centres in the network for the training of manpoer and diffusion of microbiological knowledge.

The first development in the World Network of Microbiological Resource Centres was the development of the World Data Centre for Microorganisms (WDCM), in 1975, at the University of Queensland, Brisbane, Australia. The WDCM houses a master copy of the World Directory of Collections of Culture of Microorganisms and serves as a pivotal point for fostering development of culture collections in developing countries in an international cooperation (Da Silva 1986).

To help provide permanency and continuity to the work of the WDCM, the World Data Centre, has been relocated, from July, 1986 to the Life Sciences Research Information Section, RIKEN, Tokyo, Japan.

Today, the World Network of Microbial Resources Centres (MIRCENs) is comprised of the BNF and Biotechnology MIRCENs. Culture collections continue to occupy an integral role in the network and the emphasis is more on strengthening current assets rather than funding new ventures.

To return to the theme of mycology, it is evident that there is a distinct need for basic training especially at research sites and institutions in the developing world. Emphasis to date has been on specialized training at established institutions in the developed world. Important and valuable as training is, there needs to be a concerted effort that focuses on the awareness and beneficial inputs of the mycological sciences in improving the quality of life and by consequent the environment. Emphasis should be on responding to the needs for food, health, and energy coupled to the adaptation rather than the adoption of new skills and techniques for use by researchers in the developing word.

Fungal collection such as that of the Mycothèque de l'Université Catholique de Louvain and the IMI MIRCEN have important roles to play in taking the message *into* developing countries by organizing training courses and participating as

"Professors" in these countries. Such pioneering roles help to create awareness in university administrators, help update curricula and syllabi, and help attract industrial support and participation. An example is the excellent contribution of Mycothèque de l'Université Catholique de Louvain in the development of a basic unit in the University of Burundi, Bujumbura, Burundi. The grass-roots approach has never failed. One of the best examples is the case of the study of mushrooms that has emerged on the international scientific scene as an important theme for international co-operation. In fact, these "mushroomologists" have their own society, their own computer network, their own newsletter, and their own programme of scientific activities.

In wishing the Organizers of this meeting every success, UNESCO, through its decentralized programme activities, and the Participation Programme for Member States, in which national culture collections in Bangladesh, Iran, and Paskistan have been strengthened, is ready to assist in the delivery of this message and would like, publically to thank all those scientists who have strengthen the bonds of international scientific co-operation.

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DISCUSSION

FUNGAL TAXONOMY AND TROPICAL MYCOLOGY: *QUO VADIS*?

Hennebert. It is now the time for questions and discussion. We heard this afternoon different options in approaching fungal taxonomy. In regard of the need developing tropical mycology, we may question what kind of approach is now suitable.
Dr Kurtzman and Dr Blackwell showed that molecular analysis can provide reasonable answers to problems left by the so-called classic - meaning morphology-based – taxonomy. But they made clear that such answers, if sometimes leading to definite nomenclatural changes in yeast taxonomy, are most often indicating new relationships between taxa that need further investigation before definite nomenclatural changes are made like in the Ascomycetes.
In another hand, Dr Rammeloo and Dr Roquebert pointed out the fact that the two century-old taxonomic framework of classification of the fungi is morphology-based, and that is more practical to build on it or revise it that to construct another one *de novo*. Dr Rammeloo also indicated the particular need for more precision, consistency and coherence in morphological criteria and their expression. Dr Roquebert showed promising perspectives in that direction with new types of microscopy that may diminish subjective interpretations.
For mycologists wanting to cover as soon as possible the largest part of the still unexplored and disappearing fungal diversity in mainly tropical areas, the question of methodology is surely justified. Mycologists in those areas have not so many facilities, as we heard from Dr Masuka and Dr Peerally. They are two taxonomic approaches to fungal diversity. Which of these two taxonomic approaches should they adopt? What option should we propose? Your opinions are welcome.

Webster. Let me ask Dr Kurtzman and Dr Blackwell if they feel that we are here approaching a finality in ascomycete classification whether we are moving to a stable state or we are moving to a phase that would become to develop much further.

Kurtzman. That is a very good question actually. I would like to think, on the basis of ribosomal RNA and rDNA sequence comparisons, that we are placing our systematics in a framework that would not change too much. And we have seen from comparing these molecules with other molecular sequences that they are giving pretty much the same outline. That is what we see now, and what we will see for some time.

Blackwell. I agree with Dr Kurtzman. Some people have expressed that they believe that some of these advances have been so fast that they have not been considered using ribosome genes, and it does not seem that other genes could do it. This may be pessimistic, but we have not studied enough taxa to know this. I think we are coming very soon to something quite stable for most of the Ascomycetes. Also I tend to be taxonomically very conservative, and I don't like to make taxonomic changes at this point to confuse the issue for a time.

Hennebert. At what level do you see this?

Blackwell. At the order level, for now, but later, at other levels.

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Hennebert. It seems that also morphologists are waiting to define orders within the Ascomycetes.

Blackwell. The things that we have been doing and in what we believe, is that in the Ophiostomales there has always been the same ravel. The genes we have done so far gave the same story. And if you sequence a gene that is evolving very quickly, it might be you will not get the same story. But, you will get a lot of noise that you may not be able to distinguish it. We are making gene trees. By doing that, we are making a lot of headeway from what has been made.

Hennebert. But doing such an approach is the classical taxonomy keeping some reference?

Blackwell. In my work certainly. I am a classical taxonomist, you know for thirty years. I am using morphology but it sometimes come to a certain point that I cannot decide what is right or wrong. Then I look for gene analysis possibly to provide an answer.

Prillinger. I think it is absolutely right that there is a necessary combination of classical taxonomy and systematics with molecular analysis, especially taking into account that there is in molecular biology a dependence on applied computer programs. Indeed it is possible that depending on the program we use we get different trees, so we have trees. Another aspect is that we need other characters, other methods to prove our results. It is therefore absolutely necessary for all people working on systematics not to use one method only either molecular analysis, or morphology, or anatomy, but to combine theses methods.

Hennebert. There are indeed many different programs giving different trees and neither the molecular biologists nor the fungal taxonomists know really how those programs are constructed, on the basis of what algorithms, and what is the significance and real value of these algorithms for our taxonomic purposes.
  Perhaps, and that is not a joke, mycologists should be good computer scientists, and not only good biologists, and, I should add, good molecular biologists. Therefore, if things are such, the required training to become a mycologist should change and be much broader.

Whalley. I have a question for Meredith Blackwell. Meredith, do you remember the paper by Gowan and Vilgalys (1991, Am. J. Bot. 78:1603-1607). They looked at *Xylaria magnoliae* and they looked at the ribosomal length polymorphisms from different stromata taken from the same fruit and from the different fruits in the same region and then from different localities. And they got quite a lot of variations in their data. In fact they were getting more variations, I think, on the DNA material that they would on the classical morphological characters. The question I ask is how much molecular data do you need to be confident in the results?

Blackwell. It all depends. I do know the opposite problem too, with little rDNA variation within large specimen variability. If I consider the biology of the organism, questions are how is its dispersal, how it is getting here or there. If you count its

population, is it really a population? And what is an effective population size? All these things ought to be known before being able to interpret the molecular data. Does it help?

Whalley. Thank you, Meredith. It does indicate that interpretation of molecular data must be done with great care.

Prillinger. I feel there is also different information from ribosomal DNA. If we look to a recent paper of John Taylor and Berbee, and to your paper of today, Mrs Blackwell, and to the paper of Dr Kurtzman, we have different interpretations of the Endomycetes. For John it is a side group of the Ascomycetes near the filamentous ones. In the Kurtzman paper it is a primitive group of fungi. What do we have to believe?

Blackwell. What I think of the Endomycetes? I think they are sister taxa to the filamentous Ascomycetes, do'nt you? It is somewhat deceptive when you have different arrangements from the tree made from different numbers of taxa. But I think now, looking with clean eyes, that, with the increasing number of species included in the trees, we see that the Endomycetales and the filamentous Ascomycetes are sister taxa.

Kurtzman. These are indeed not far away.

Blackwell. Dr Sugiyama what do you think?

Sugiyama. I agree with Dr Blackwell. From phylogenetic analysis of the nuclear small subunit rRNA (18S rRNA) sequence data, we found three major lineages within the Ascomycetes. their distinction supported by very strong bootstrap confidence. These are the Archiascomycetes (early Ascomycetes), the Hemiascomycetes (yeast Ascomycetes) and the Euascomycetes (filamentous Ascomycetes). The Archiascomycetes comprise *Taphrina*, *Protomyces*, *Saitoella*, *Schizosaccharomyces* and *Pneumocystis*. The Archiascomycete lineage diverged first among the ascomycetes, whereas the Hemiascomycetes and Euascomycetes as sister taxa evolved more recently.

Korf. I like to shift a little bit from phylogeny and relationships of organisms, which is indeed an example of the most exciting things that come out molecular work, to look, in fact, at "fungal taxonomy and tropical mycology: *quo vadis*?", as our topic today, specifically at biodiversity and particularly at biodiversity in the tropics.
  The question I want to rise is how to go about training fungal taxonomists. That is mostly only done in temperate areas by people that have been trained in temperate areas also. My own experience with both temperate and tropical floras had led me to realize that we have that tremendous history of a development of morpho-anatomical systems primarily based upon temperate floras.
  When I went to the tropics, I discovered something that made me change all my viewpoints upon how one has to train a mycologist and I always insist on, and force all my students to collect in the tropics. It is not only that the tropics are loaded with a lot of species, and we know from the biodiversity information that the number of species in the tropics may be usually in excess of that in the temperate areas, and

that they have been vastly understudied, as has already been said several times today. What really strickes me as most amazing was that my generic concepts were immediately challenged the moment I moved to the tropics. I kept finding things that were neither this nor that, and when I hear of my colleagues here saying, we want to identify at least to genus, I do not know how you can, given the way current taxonomy is arranged. There are temperate genera. If you are going to work in the tropics you will have to find that you are going to have all new systems, you are going to have to develop them. And you are going to find lots of species that you cannot put names on.

Now, I spent all my life travelling all over the world convincing the dean of my College that I could not possibly study the fungi in Ithaca, New York, without going to the Canary Islands, to Africa, to Asia, Not true? I could spend all my life studying the fungi outside my door and I would still not have had all the answers because I don't know two-thirds of the fungi outside my door. But the tropics really change your perspective. And I am sorry for the fact that there are not many places in the tropics where taxonomy is being per persued today, and if it is going to be persued it has to be persued with the idea that the tropics have lots of unanswered questions, lots of things to be described, and that we indeed must go there and collect these things. Rammeloo, Masuka and Peerally have clearly shown that the traditional morpho-anatomic methods are what is going to have to be done if we want to document what is there, and to go and collect them in order to explore that tropical flora.

Where we are going to find the mycologists being trained to monograph large genera, or to find financial support for people producing large monographs? That is a big and unsolved problem. And I want to sound an alarm. An alarm that does not seem to have been voiced today. We must stress, no matter how exciting molecular techniques are, and they are exciting - and if I was a young student again, I am sure I would be a molecular taxonomist - they *will not, and cannot,* address the problem of biodiversity or cataloguing of our tropical floras.

In addition, classical mycologists are losing out in these areas. When I look about me, I see mycological taxonomic positions in numerous universities all over the world, in museums, being replaced by either nobody or by molecular taxonomists whose interests are not in producing monographs, or in cataloguing the tropical floras that we know so poorly.

I just wanted to point out my worries for the future of our understanding of biodiversity in the tropics.
(Applause.)

Webster. I truly share what Dr Korf has said.

Hughes. Grégoire, it was a surprise and a pleasure to be invited here. Yes, in order to survive the Ottawa winters, I have been a long time in the tropics. When I think of the tropics, I think of the tremendous leaf surface of the living leaves, and probably in these land areas where the leaves and the nutrient within are separated from the fungi outside by a thin cuticle only.

I stated as a novice at IMI in 1945. In 1946, Hansford's wonderful work on 'Foliicolous Ascomycetes and their parasites' appeared. The availability of this work and the riches in herbarium IMI, followed by a 3 month foray in Ghana in 1949

further impressed upon me the enormous microfungus diversity even in the small area that I covered.

Flat leaf surfaces in the tropics support a vas array of Ascomycetes. For the most part theses have been assigned to well over 30 families in various orders. Some of the fungi are entirely superficial and comprise several families of the pleomorphic sooty moulds. Others are leaf parasites and, as Hansford has stressed, various devices have evolved to tap the nutrient resources of the leaf. These fungi can cause severe damage or may be benign parasites which cause minimal damge to the leaves, such as the Meliolaceae, Asterinaceae and several other families. A third group of common Ascomycetes comprises the mycoparasites of other foliicolous fungi.

But the numerous families of foliicolous Ascomycetes are by no means well defined. There is a great need of studies on the hyphal behaviour on leaf surface, perithecium ontogeny, development morphology of the type species as well as the identification of thei anamorphs. Such studies can be carried out adequately with ordinary microscope using current simple and straightforward techniques. We have much to learn about, and from these beautiful Ascomycetes.

Subramanian. I am entirely agreeing with much of what has been said here. I feel really happy that we are going to make great progress in the field of molecular taxonomy. But I think, for the developing countries, which don't have a complete inventory of their fungal resources, they have to rely on the ordinary light microscope and the simple tools which are at their command.

And, to begin up, the collections and the herbaria from those regions, which remain largely unexplored, have to be done by the people in these regions. Such a task needs, of course, cooperation and help which they can get from mycologists in the advanced countries.

Just to make an example. how pertinent observations can be made by ordinary light microscope, my good friend Stan Hughes here, his work in his 1953 paper on conidiogenesis and the classification of the Hyphomycetes was based entirely on observations under the ordinary light microscope. And so was it earlier with Vuillemin, Mason and other people. And we do know now how much of it has been confirmed by studies with better tools, like the transmission electron microscope, the scanning electron microscope and, maybe, studies on the biosynthesis of certain metabolites may also confirm some of these things.

The important thing to do, is to use the ordinary light microcope, the phase contrast microscope, in the best way, to study the fungi. They are so beautiful. They are everywhere. Professor Korf said there are so many new things in the tropics. Almost everything is new! But of course it is also interesting to see the old fellows when you collect. Some beautiful things also occur in the tropics that are in the temperate regions.

I think we ourselves must define our *priorities* in developing countries. And the first thing to do is the *inventory* of the fungi using *simple tools* which may be easy to get, the ordinary light microscope, and the literature. Getting past literature is more difficult. But there again, things are better now. Things were not the same when Saccardo compiled his *Sylloge fungorum*, we have better things today to come up with lists of fungi. So I thing the first priority is the inventory.
The second priority is to have *monographs*. This has been expressed already.

I believe it is up to us, people of the developing countries, to do this job. And I am sure if we seek for cooperation of the mycologists in other countries, we will get it, as I got it when I started my work in mycology.
(Applause)

Parmasto. I wish to comment about the importance of collecting and use of tropical specimens.....

First, you make a data matrix, trying to make a system of the species you have classified using phenetic or cladistic methods. Then you are adding data and many other species and your cladogram is almost totally changing. That means that we not only have need to know more species of the tropics, but also more various collections of each species.

Secondly, if nowadays there are not many possibilities to use molecular methods in tropical countries, it is possible to collect specimens in such a way that they will be usable in future for such studies. That means that specimens must be collected and preserved in such a way that their DNA are not damaged, f. i. by chemicals, high temperature, or simply contaminations..As far as I know no one has been using well preserved spore print on cellophane for molecular studies. One spore is enough for sequencing. I hope that somebody shall do it and tell how it does work.

Third, it concerns molecular data as well as morphological ones. The molecular data are mainly based on morphological classification of the species. They are meant to check each other, inducing revision of the morphological classification, but also revisiting the exact value of the numerical treatment of sequences. Only after that process, they are to unite. It seems that it is not so important how to make the sequencing. Half of the work comes after sequencing, how to use the data. An seen on the posters, they are nice cladograms, made using neigbour-joining method. When using cladistic methods, instead of one cladogram, one shall get 3466 cladograms of equal length. That implies that training young taxonomists must include such courses as philosophy of sciences and methodology of sciences. We are a little bit too old, including me, to understand what to do with all the data we have, but let them have a little more "soil under their feet", a better background to understand what they do.

The last not the least, seventy to eighty percent of new fungus species are based on one collection. The percentage of one-specimen species has been very stable for the last forty years. As a result, description of such new species does not give any information about the variability of the species. Half of these new species may never been found later again. Because they are specimens but not real species, they are producing noise in data treatment. We should avoid that situation.
(Applause)

Masuka. Yes, it was good to get some corroboration on the most important things to do, that is to go out, to collect, to document and to make *inventoies*. That is priority for us. We accept, of course, what temperate scientists are saying on molecular taxonomy.

But wa are convinced that the priority is finally to compile check lists of the fungi. We know that check lists form the base for our studies. Then, of course we must do monographs.

An important thing that was hightlighted is that, as Professor Perally said, in our work agenda in developing countries we must seek for collaboration and cooperation

with laboratories in the developed world. That has been so in the past and that must continue.

Arnold. I would like to indicate that only one existing system of the fungi is a complete one, the one of Saccardo, contructed on the base of all fungi known at his time. All the following systems are incomplete, as constructed on only a part of the fungi: Ascomycetes or Basidiomycetes or Fungi Imperfecti. If one wants to make a system on the basis of molecular data, one must study about 75000 existing species. You can imagine how many years such a work needs..
We have to combine all data on morphology, anatomy, biochemistry of these species with the data of molecular biology. This would make a system, may be almost natural.

Hawksworth. I feel the time has come to startt thinking in terms of different practices. This is an issue that has been brought up many times over the years, and initially by the mycologist J.H. Corner in 1946 who, having to cope with a tropical flora of not only the fungi but the trees that are unknown, decided to monograph the trees first, viz. the two volume 'Trees of Malaya'. Then he was able to continue work on the fungi, something he is still doing. Corner's way of operating, drawing carefully all that he saw, led him see things that others had not seen before, often because they were looking with temperate trained eyes and with preconceived ideas. A fresh look is very valuable, and a great deal of the work can be done locally.
   We also have to consider making data available. Dr Huhtinen, at the First Intensive Workshop on Ascomycetes Systematics, in Paris last year, pointed out that also we have to think more about data capture, as well as trying to get fungi into culture close to the site of collection while the specimens are still fresh. With the resources we get, we can carry out the other work, make comprehensive drawings, look at all possible characters and determine ways to exchange these data electronically. We have been thinking about this at the IMI. This is also true in the insects. We can not think just about conventional publications, as the volume of data will be so great. In the case of insects, it has been estimated that something like 1-6 km of shelves would be needed to accommodate the literature on the Earth's insects at the rate of two pages per species. The task is absolutely enormous, so we have to develop new ways of working.
   In the tropics, this means really developing a taxonomy as we go along. Dr Subramanian, Dr Korf, and others have mentioned today that a vast number of fungi are unknown. So it is not a matter of working with a key that exists, because we will find very quickly a huge number of genera, even of families, not documented in the keys. The same phenomenon arises in specialized habitats even in Europe, as some of you will know.
   What I have been very pleased to see florishing in the IMA, is the regional committees. This is has been a noticeable development, especially in the African Committee, that we have been hearing about today: two major conferences have been held, a third one is being planned next year, together with a training course, a new journal has been launched, and an African Center of Biotechnology and Ecology in Cairo has recently managed to secure almost two millions dollars to actually set it up.
   This move should be linked with other programmes that have been mentioned several times today. There are entomologists meeting at this very moment 20 km

from here in Belgium looking at the development of BioNET INTERNATIONAL in Europe. BioNET is a concept that CAB INTERNATIONAl, as a peculiar intergovernmental organization mainly owned by developing countries, has developed in agreement with major institutions around the world, particularly in North America and in Europe, to establish a number of regional biosystematics loops which bring together the expertise in different regions. It is amazing how many specialists there are in now developing countries, but who don't necessarily know each other at all, being in different departments of a university or in different institutions.

This networking can be linked with a building up of collection resources locally, and there is money for this type of activity to be sought. I mentioned the year 1995 as a critical date for planning in mycology, in a paper I gave in 1992. That date still stands, as it will be the watershed as to how the funding of the Global Environment Facility will be used. The fund has a large amount of money available for biodiversity and related actions, with the 2.1 billion dollars so far pledged to this fund. The fund is being re-structured in November 1994. Mycologists need to think how we actually tackle that funding source. And because the fund scale is so huge, you cannot tackle it just as mycologists. We have to present ourselves as systematists, linking with other groups.

The major problem is to increase recognition that, in order to support the development and strengthen country capabilities, the training of people in the developing countries is visible. Yet it is up to them to work on their priorities and make their submissions for support. IMI has been fortunate in securing biodiversity related money under the Danish Initiative scheme, not just for mycology but including entomology and parasitology. Through this initiative, 22 fellowships for systematists with some training are already established in positions in developing countries and founded. Money is around if the right sort of proposalsis is made and if we think much more as biologists and systematists, rather than only as mycologists.

Demoulin. Just a small point, I wish would not be forgotten concerning tropical mycology. Is it true that we need to put inventory as a priority? There is also a thing, perhaps more experimental, but within reach of many laboratories, including those in developing countries, which is quite interesting. It is ecophysiology and autoecology of the species and of the strains and the study of the differences between the tropics and the temperate zone. Just to give an example, with a case that is studied in Papua New Guinea by several Belgian laboratories. We found the North-temperate *Polyporus squamosus* in Papua New Guinea. The species has a well defined North-temperate ecology concerning the optimum temperature. Although it is definitely not well adapted in that tropical area, it is still fruiting there. There are many similar cases that can be studied in an ordinary laboratory, with not much equipment.

Hennebert. I shall try to conclude in few words. It comes out the discussion particularly, that there is a priority on a classical approach of the large unexplored diversity of fungi in tropical countries. A priority and urgent duty is to describe and make the inventory of this mycoflora, using microscope and culture *in situ*, for which well trained young mycologists are needed. And on that basis, elaboration of monographs should provide the necessary tools to further investigations.

This does not at all exclude research in molecular taxonomy, to solve taxonomic problems, afterwards. There are lots of problems arising every day in fungal taxonomy in classifying tropical fungi. As Dr Korf and Dr Hawksworth said, when collecting in tropical countries, we need almost to forget our temperate system of fungi and be open to construct new concepts. Taxonomy is an evolving field where revision is current.

Moleclar taxonomy does not supercede morpho-anatomical taxonomy. On the contrary, they are complementary to each other. Good phylogeny based on molecular data may demonstrate relationships that might lead one to amend the system of the fungi at the highest ranks. But the molecular approach has not opposed the species concept as defined by classical taxonomists as wrong.

Another issue from this discussion is the important need of a new training profile with collaboration from both tropical and temperate regions. That training should cover different disciplines, including computer science, cladistics, molecular biology, ecophysiology, without neglecting at all the traditional but unavoidable and basic methodologies of fungal taxonomy: collecting, microscopying, drawing, isolating fungi into pure culture, studying type materail, interpreting past literature, writing descriptions, constructing keys, writing monographs, building expert systems of identification and managing herbaria and culture collections. These are the first acts for a mycologist in tropical and unexplored areas of the world.

Thanks to all of you for your contribution.

The founders of the Mycothèque MUCL:
Pr. Jean-Baptiste Carnoy (1894), Pr. Philibert Biourge (1894-1934), Pr. Paul
Simonart (1934-1964).
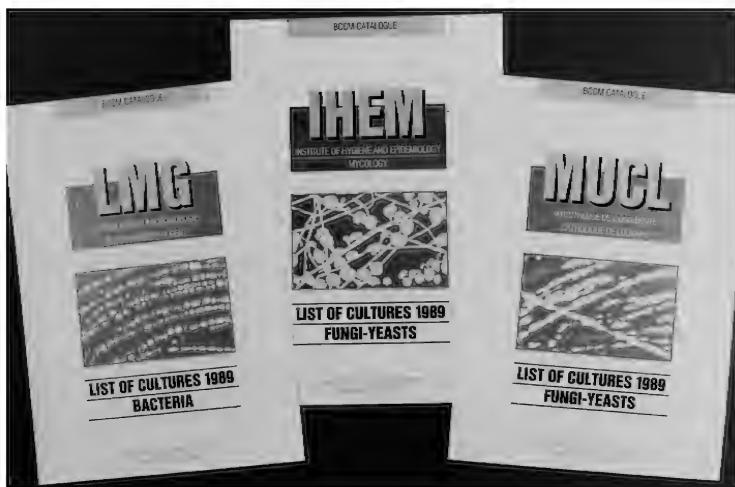
The first public catalogues of the three Belgian Collections of Microoganisms
coordinated in the BCCM (1987)

Pr. Drs D.L. Hawksworth (IMI, UK), D. van der Mei (CBS, NL), M. Blackwell (MSA, USA), C.P. Kurtzman (NRRL, USA), M.-F. Roquebert (CP, FR), J. Rammeloo (BR, BE), A.J. Masuka (FRC, Zimbabwe), A. Peerally (MU, Mauritius), J. De Brabandere (SPPS, BE), P. Rouxhet (Prorecteur UCL), H. Naveau (Dept. Head, UCL) & G.L. Hennebert (UCL)

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Drs (1) Hughes, (2) Cerisier, Kersters, Korf, Gams, De Brabandere, (3) Webster, Blackwell, van der Mei, (4) Masuka, Roquebert, Subramanian, Sugyama, Spencer (5) Mrs Korf, Mrs Hennebert, Hawksworth, (6) Martini-Vaughan, Cimerman, Uruburu, Parmasto, Fraiture, (7) Maraîte, Kurtzman, Marson, Martini-Vaughan, Uruburu, (8) Verbeken, Walley.

Fig. 1. View on the exhibition of cultures from three classes of fungi, original drawings of Hyphomycetes, catalogues of culture collections and publications.
Fig. 2. Ancient equipments and manuscripts from Pr. Dr Ph. Biourge.
Fig. 3. Evidence of the MUCL activity in applied mycology.

Openair lunch with breads, cheeses and beers from Wallony, at the Faculty in
Louvain-la-Neuve: Figs. 1, Dr Sughyama, Dr.Hughes; 2, Dr Hughes, Dr Korf;
3, Dr Peeraly, Dr Hawksworth; 4, Dr. Vanderveken; 5, Dr Nguyen, Hennebert,
Dr Parmasto; 6, Dr Walley, Dr. Kersters, Dr.Spencer.
7-8. African concert near the ruins of the Abbey of Villers-la-Ville

The lunch, at the Faculty in Louvain-la-Neuve

The diner, in the Abbey hôtel of Villers-la-Ville

The Faculties of Agronomy and of Biological Sciences, Louvain-la-Neuve

The Laboratory of Systematic and Applied Mycology (MUCL)

POSTERS

FUNGUS COLLECTIONS AND SERVICES

THE MYCOTHEQUE OF THE UNIVERSITE CATHOLIQUE DE LOUVAIN (MUCL)
100 years 1894-1994

GREGOIRE L. HENNEBERT

MUCL, 3, Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

The genealogy of the MUCL culture collection is presented. Under the pulse of Carnoy, Philibert Biourge inaugurated his young collection of yeasts, molds and bacteria in 1894. He left the hand to his young colleague Paul Simonart in 1938 Simonart named the collection "*Mycothèque Philibert Biourge*" in 1939. He maintained the collection untill 1969, when he passed it to the author.

Jean-Baptiste Carnoy
(1836-†1898)

Fr. Dierckx →	**Philibert Biourge**	← A. Cappuyns
R. Mosseray →	(1864-†1942)	← E. Wieldersz

1894-1934
Penicillium-Aspergillus
yeasts (kept in School of Brewery)

Paul Simonart
(1907-†1997)
1934-1969
Mycothèque Biourge   ← 1949-1960   Raymond Lambert
300 strains

Joseph Meyer 1960-1969 →
(1924-20--)
1950-1960
Fungi of Congo
450 strains

Grégoire Hennebert
(1929-20--)
1956-1969
*Fungi of Europe
and North America*
3200 strains

Grégoire Hennebert
1969-**1994**
Mycothèque de l'UCL
MUCL
1972: UCL-IATP-WFCC
1983: BCCM – ECCO
1985: MINE
35000 strains

Hennebert integrated the Biourge's Collection with his own one into the new "*Mycothèque de l'Université Catholique the Louvain*" internationally recognized by the IAPT in 1969 ant the WFCC in 1972. MUCL, in 1983, became one of the two first collections of the *Belgian Coordinated Collections of Microorganisms* BCCM and a member of the European Culture Collection Organization ECCO. In 1985, it is a member of the Microbial information network, and in 1992, an International Depository Authority IDA under the Budapest Treaty.

The Collection made important contributions to fungal taxonomy (François Dierckx in *Penicillium*; Philibert Biourge in *Penicillium*; René Vandendries in *Basidiomycetes*; Raoul Mosseray in tropical *Aspergillus*, Paul Henrard in tropical *Xylaria*, Pr. Joseph Meyer in African soil Hyphomycetes, ...Others developed applied mycology and biotechnology: Ernest Wieldiers, the Bios production by yeast; Alphonse Cappuyns, the citric acid production by *Aspergillus niger*, Pr. Paul. Simonart: the discovery of the griseofulvin among metabolites of *Penicillium griseofulvum*; Pr. P. Simonart and Pr. R. Lambert, the quality control of dairy products and the invention of the milk aseptisation by ultracentrifugation. From its start, the collection has been oriented both to fungal taxonomy and to applied mycology.
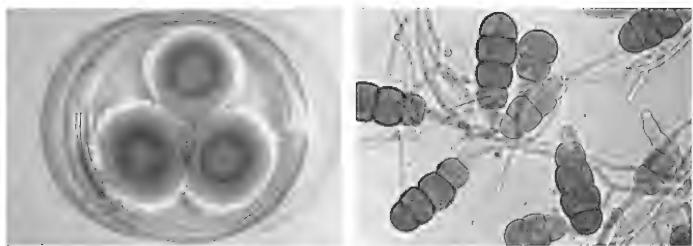
MUCL, a Public Service Collection

C. DECOCK, CH. MOULLIARD, L. NELISSEN, P. MASSART, P. EVRARD, N. JAMIN, P. CHARUE AND G.L. HENNEBERT

Mycothèque de l'Université Catholique de Louvain, Unité de Microbiologie, Faculté des Sciences Agronomiques, Place Croix du Sud 3, B-1348 Louvain-la-Neuve, Belgium

The MUCL culture collection is a partner of the Belgian Coordinated Collections of Microorganisms (BCCM), the four complementary collections of fungi, yeast, bacteria and plasmids in Belgium. Besides the mycological research, MUCL has developed many services for the scientific and industrial community. The Mycothèque MUCL is indeed recognized as a public service collection. Its main characteristics are the field collection, identification, isolation and preservation of a constantly increasing patrimony of fungal strains (yeast and fungi), of taxonomic, environmental, (agro)-industrial and biotechnological interest.
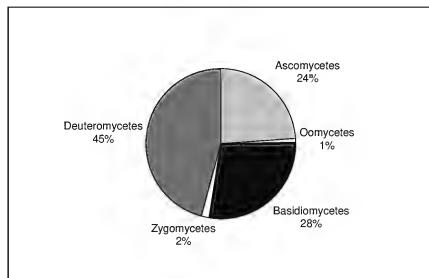
As public collectiopn, MUCL is aimed to offer many confidential services (Anomymous 1973, 1994) such as:
1) Identification of yeast and fungi by means of morphological, physiological, biochemical and gene-sequencing analysis. Isolation and identification of fungi from submitted products.
2) Material deterioration and material testing for resistance to fungicides and bioassays of fungicides according international standards.
Survey and expertise of fungal decay and mycoflora in buildings and industries, with recommendations of contrôl.
3) Fungal analysis of agricultural and industrial food products.
4) Screening and characterization of strains with particular properties, and creation of strains with specific enhanced property.
4) Distribution of strains. Biomass production;
5) Patent deposit under the Budapest Treaty. Confidential safe deposit of industrial strains. Deposit of strains for the MUCL public collection.
6) Training in general, taxonomic and applied mycology with special attention to undergraduate and gradute researchers from abroad.
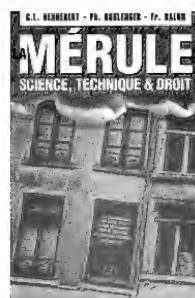
The MUCL collection currently holds thirty thousands of strains of filamentous and yeast fungi of all groups especially *Zygomycetes*, *Ascomycetes*, *Hyphomycetes* and *Basidiomycetes*, and includes taxonomically and biotechnologically important reference strains. Since 1992, the 2d edition of the MUCL catalogue containing 7300 strains of filamentous and yeast fungi is available on request at MUCL. A particular attention has been put on the incorporation of reference strains for industry, for identification and for bioassays and material testing.

Anonymous 1973. Laboratoire de Mycologie systémùatique et appliquée. Revue des Fermentations et Industries alimentaires 28(2): 80-81.
Anonymous 1994. La Mycothèque de l'UCL a cent ans. Champignons: un siècle de passion. UCL Quinzaine universitaire 36: 1.

P.S. In 1998, a third edition of the MUCL List of Cultures was issued with 8300 selected strains of fungi.

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MUCL, a Fungus Culture Collection,
a service to African fundamental and applied mycology.

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It is the aim of the MUCL fungus culture collection to make its expertise in fundamental taxonomy of fungi and in aspects of applied mycology available to serve African countries. Such a contribution could be offered by means of ⬚ eveloped⬚ ence, exchange of materials, training and research assistance *in situ* in Africa, training and research periods at MUCL. Training and research grants are available from the Lomé agreements, by the GD VIII and GD XII of the ECC, by the National Agencies for Cooperation and Development of Switzerland and Canada, and by international arrangements for higher education and development such as Association des Universités Partiellement ou Entièrement de Langue Française AUPELF, Association des Universités Africaines, International Foundation of Science, International Development Research Center, United Nations University, World Bank, FAO, UNESCO and MIRCEM, and from Belgium by the General Agency for Cooperation and Development and by the Commission for International Cooperation of the Catholic University of Louvain.

MUCL has been collaborating with the University of Burundi, Faculty of Agricultural Sciences, Bujumbura, in establishing a Laboratory of Microbiology mainly oriented toward food microbiology.

During the last decade, the Laboratory of Microbiology LMUB (see photos) has carried out, owing national and international financial support, several projects for food sanitation and quality.

- Fungal deterioration of cereals and legumes in storage in Burundi (CEC)
- Improvement of storage conditions for cereals and legumes in Burundi (AGCD).
- Microbiological quality control of dried fish ndagala from Lake Tanganika (FED)
- Selection of an appropriate drying technology for optimal microbiological and sanitary quality of dried fish (UB)
- Quality control of indigenous and imported food products as a current service of the Laboratory LMUB since 1985.

MUCL is also ⬚ eveloped research project in applied mycology for the Tropics, particularly on food production with different African countries. With Brazzaville and Matsoumba, Congo, MUCL investigated the solid fermentation of cassava by

Rhizopus oryzae for its protein enrichment. With Guinea, Zaïre and Cameroon, diverse levels of collaboration, guidance, local experiments and PhD research, concerned the domestication of native species of edible mushrooms, the evaluation of local wastes as cultivation substrate and the improvement of yield and protein content by appropriate substrate composition (Tshinyangu 1994).

The production of mycorrhizae is another field of interest for the Tropics. Original mycorrhizogenic fungi, like *Suillus cothurnatus* from Perou, *Pisolithus arhizus* from different continents, are cultured and used in the mycorrhization of *Pinus spp*, both in laboratory experiments and in the field for reforestation.

Also MUCL provides skill and training in many aspects of fundamental and applied mycology. In fungal taxonomy, it offers training in isolating, identifying, describing and publishing local fungi. MUCL has contributed to the development of an African Culture Collection of fungi and yeasts at the Laboratory of Microbiology, University of Burundi, at Bujumbura. The Collection LMUB contains more than 800 strains. Duplicates are deposited in MUCL.

Robert *et al.* (1994) have set up an expert programme for the identification of yeasts, which has been elaborated and tested on a large collection of yeasts isolated by the first author from Burundi.

MUCL, for the I.M.A. International Committee for the Development of Mycology in Africa, and with the financial support of the Centre Technique de Coopération Agricole et Rurale ACP-CEE Convention de Lomé, has published the first Directory of African Mycology, a tool that should enhance mutual contacts and collaboration between African mycologists themselves and with non-African mycologists.

Ndenzako C. 1986. Les altérations fongiques du Sorgho en cours de stockage. Cas de la provinde de Kirundo, Burundi. Université du Burundi, Bujumbura, 143p.

Ngabonziza P. 1987. La croissance des moisissures de stockage et l'activité de l'eau. Le genre *Aspergillus*. Université du Burundi, Bujumbura, 59p.

Simbizi J. 1987. La contamination fongique du poisson séché au Burundi. Université du Burundi, Bujumbura, 68p.

Nyandwi A. 1987. Contamination fongique du Haricot avant et après la récolte: recherche sur la flore du stockage. Université du Burundi, Bujumbura, 110p.

Baert W. 1988. Valorisation de la Bagasse de Canne à sucre par la production de Pleurotes en Côte d'Ivoire. Fac. Sc. Agronomiques, UCL, 52p.

Tshinyangu K. 1994. Production et valeur alimentaire du Pleurotes en fonction du substrat. PhD thesis UCL, 255p.

Robert V.,De Bien J., Buyck B. and Hennebert G.L. 1994. ALLEV, a new program for computer-assisted identification of yeasts. Taxon, 43: 433-439.

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THE ACTIVITIES AND MYCOLOGICAL COLLECTIONS
OF THE BELGIAN NATIONAL BOTANICAL GARDEN (BR)

ANDRÉ FRAITURE

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The study of African Fungi

The first works of African mycology published by the Botanical Garden were edited by É. De Wildeman and T. Durand, who were both directors of the National Botanical Garden in Brusseles (Bresadola and Saccardo, 1899; De Wildeman and Durand, 1901; De Wildeman 1905-1907; De Wildeman 1903-1912). The study is about collections which were carried out by various collectors (principally A. Dewèvre and H. Vanderyst) and identified by J. Bresadola, P. Hennings, P.-A. Saccardo, and H. and P. Sydow. This materiel consists of more than 450 taxons of which close to 400 are new to mycology.

The first mycologist from the Botanical Garden to have worked systematically on the collections of African fungi was Maurice Beeli (1879-1957), who worked there as a scientific collaborator for more than 30 years (Heinemann 1959). Although never having stayed in Africa, he worked extensively to develop the study of the mycoflora of this continent. Following on his advice, Mme Goossens-Fontana started colleteing from 1919 numerous specimens for the Botanical Garden, while also adding notes and watercolours (more than 900). This collection constitutes a remarkable source of knowledge about African fungi. Between 1920 and 1940, M. Beeli published close to 30 studies on the African fungi preserved in the Botanical Garden, 11 of which are recognized in the "*Fungi Goossensiani*".

The efforts of M. Beeli to highlight these collections succeeded in the publication of the "*Iconographical Flora of Fungi from the Congo*", the 17 volumes of which were published between 1935 and 1970. This flora was enlarged by the "*Illustrated Flora of Central African Fungi*", the 16th. volume of which is in the course of publication. It presents descriptions and identifcation keys, accompaigned by line drawings and colours prints, for differents groups of Basidiomycetes, Ascomycetes, and Myxomycetes. Several Belgian (P. Heinemann, M. Beeli, B. Buyck, J. Rammeloo, D. Thoen) and foreign mycologists (J. Boidin, E.J.H. Corner, R.W.G. Dennis, H. Dissing, R. Heim, E. Horak, M. Lange, M. Le Gal, R.A. Maas Geesteranus, D.N. Pegler, H. Romagnesi, R. Singer, R. Watling) collaborated in this work.

The presence of R. L. Steyaert (1905-1978) (Bienfait, 1979) at the Botanical Garden also needs mentioning. He conducted the greatest part of his career in Africa, then at the Commission for the Study of the Flora of the Belgian Congo and Ruanda-Urundi. He was interested in African phytopathology and studied primarily the genera *Pestalotia*, *Monochaetia* and *Ganoderma*. He left an abundance of material at the Botanical Garden.

The Study of European Fungi

From the second half of the last century, collaborators with and personnel of the Botanical Gardien became interested in the mycoflora of Belgium. É. Bommer (1832-1910) (Rousseau, 1910) and E. Rousseau (± 1845-1926) gathered a great deal of materiel for the herbarium and published several floristic catalogues. Paul Nypels

(1865-1909) was interested in species of harmful plants. Émile De Wildeman (1866-1947) (Robyns, 1948) carried out the cryptogamic part of the "*Prodrome of the flora from Belgium*" (De Wildeman 1898, 1899).

During the first half of this century, Beeli published several works and numerous notes concerning the mycoflora of Belgium. In the 1960's, Steyaert published several articles on the *Ganoderma* of the European flora.

The series "*Icones Mycologicae*" debuted in 1982. It presents high quality illustrations and detailed descriptions of various species of fungi. In this same year, 130 species, mostly European, were described in this way.

The distribution of various species of fungi was studied as well. The "*Distributiones fungorum Belgii et Luxemburgi*", the second volume which should appear in 1994, presents annotated distribution maps. Today, a cartography programme developed by A. Empain lets one produce distribution charts by computer.

Finally, the Laboratory conducts public service activities as well. This service consists mainly of identifying fungi and providing expertise about fungi which grow in homes. The Laboratory is also consulted by the Centre of Anti-Poisons when there are possible cases of mushroom poisoning.

The Fungus Herbarium

The Mycology Herbarium of the National Botanical Garden Botanique National consists of about 126,000 specimens. It is divided into three parts, according to the geographical source of the collection:
- the European Herbarium gathers together specimens collected in Europe and in the countries of the mediterranean bassin; it contains some 88,000 specimens;
- the African Herbarium is constituted of specimens collected in Afrique, south of the Sahara; and accounts for some 25,000 specimens;
- the General Herbarium unites the exsiccata coming from the other regions of the world, and contains about 13,000 specimens.

Among these specimens figure close to 2,500 types (1,100 for the African Herbarium, 900 for the European Herbarium and 500 for the General Herbarium). The Herbarium consists of type species created notably by the following mycologist: M. Beeli, J. Boidin, E. Bommer and E. Rousseau, H.F. Bonorden, G. Bresadola, B. Buyck, E.J.H. Corner, G. De Notaris, R.W.G. Dennis, H. Dissing, L. Fuckel, C.G. Hansford, R. Heim, P. Heinemann, P. Hennings, E. Horak, K. Kalchbrenner, P. Karsten, J. Komarov, M. Lange, M. Le Gal, M.A. Libert, R.A. Maas Geesteranus, Ém. Marchal, J. Moureau, V. Mouton, G. Passerini, C.H. Peck, D.N. Pegler, F. Petrak, J. Rammeloo, H. Romagnesi, H. Rehm, H. Riess, C. Roumeguère, L. Ryvarden, P.A. Saccardo, R. Singer, R.L. Steyaert, P. Sydow, C. Torrend, F. Von Thümen, R. Watling, G. Winter.

As well, the mycological herbarium contains several collections of watercolours representing fungi. These watercolours were produced by several artist-mycologists; the most important collections are those from Marthe Goosens-Fontana (African mycoflora) and Omer Van de Kerckhove (European mycoflora), but also those from M. Beeli, J. Bruylants, P. Heinemann, L. Imler, E. Klopfenstein, G. Malençon.

Also, the Laboratory possesses a collection of ± 6.000 slides, in addition to numerous dossiers containing notes, descriptions, and optic- and electron-microscope photographs, etc...

The Mycological Library

The Library of the Belgian National Botanical Garden contains close to 2000 books on mycology, among those which happened to be originals from Ch. D. Badham, J.B. Barla, M.J. Berkeley, A.N. Berlese, É. Boudier, J. Bresadola, J.B.F. Bulliard, C. Clusius, M.C. Cooke, A.C.J. Corda, L. Forquignon, E.M. Fries, L. Fuckel, C.C. Gillet, R. Hesse, H. Hoffman, D.G.F. Hoffmann, K. Kalchbrenner, P.A. Karsten, J. Kickx, P. Kummer, E. Lambotte, C. Linnaeus, N. Patouillard, M. Paulet, C.H. Persoon, L. Quélet, P.A. Saccardo, J.C. Schaeffer, J. Schroeter, L. Secretan, C. Spegazzini, L.-R. & C. Tulasne, G. Van Sterbeeck, D. Viviani, G.D. Westendorp, O. Wunsche. The library also contains a collection of 100 periodicals that are strictly mycological, al though not all sets are complete, as well as numerous other public periodicals that sporadically contain articles on mycology. Finally, there is also a collection off-prints.

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Bresadola J. and Saccardo P.-A. 1899. *Fungi congoenses.* Bulletin of the Royal Botanical Society of Belgium **38**(2):152-168 + 5 pl. [Collection of Alfr. Dewèvre, 87 taxons, 17 which are new; De Wildeman and Durand 1901 made numerous references to this publication calling it, "Durand and De Wildeman, Materials for the Flora from the Congo **5**: 33-48 (1899)"].

De Wildeman É. 1898. *Prodrome of Belgian Flora.* Pt.I, vol. 1-3, and Pt.II, vol. 4. Castaigne, Brussels. 543 + 160 pp.

De Wildman, É. 1899. *Prodrome of Belgian Flora.* Pt.II, vol. 5 and 6. Castaigne, Brussels. pp. 161-480.

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Heinemann P. 1959 Maurice Beeli (1879-1957). *Bulletin of the National Botanical Garden in Brussels* **39**(1):1-6 + 1 portrait.

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Rousseau E. 1910. Madame J.E. Bommer, née Elisa Destrée. *Bulletin of the Royal Botanical Society of Belgium* **47**(2):256-261 + 1 portrait.

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CLASSICAL TAXONOMY OF THE FUNGI

ANAMORPH-TELEOMORPH CONNECTIONS IN THE XYLARIACEAE

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The Xylariaceae is a large family belonging to the ascomycotina and encompassing perhaps as many as 40 genera (Eriksson and Hawksworth 1993). The family is worldwide in distribution but exhibits its greatest diversity in the tropics and subtropics (Rogers 1993, Whalley 1993). The majority of species occur on angiospermous wood but there is also good representation on leaves, fruits, animal dung, and even on abandoned insect nests (Whalley 1985). To date most species investigated produce both teleomorphic and anamorphic states.

Traditionally, but also for convenience and sometimes through necessity, the teleomorph has assumed the central role in taxonomic decision making and has been the source of data for most identification keys. There are, however, situations where a teleomorphic form is absent and under these conditions the anamorph inherits the leading role for identification purposes.

The ability to relate anamorphs with known teleomorphs is dependent on the taxon involved and the ecological situation in which it occurs. Thus in many of the Xylariaceae anamorphs occur on the immature teleomorph and recognition of the connection is readily made. In other taxa they can occur separated both in time or space and connecting the two states now becomes more difficult and uncertain. A further challenge is provided by the apparent widespread occurrence of these fungi as endophytes of a wide range of plants where in most cases identification of the fungus is limited to features of the anamorphic state (Petrini and Petrini 1985). To what extent confident assignment to a known xylariaceous taxon can be based on the anamorph alone is open to discussion although several authors have reported species distinctive characteristics which enable identification in the absence of a teleomorph (e.g. Greenhalgh and Chesters 1968, Jong and Rogers 1972, Petrini and Muller 1985, Petrini 1992).

In the case of these endophytic isolates many undoubtedly have xylariaceous affinities but Rogers (1993) urged caution in making assignations without a clear connection to a teleomorph stating "in my laboratory anamorph and teleomorph connections are considered as definite only after ascospore cultures have been established or after cultures from natural substrata have been equated with known culture types that originated from ascospores". To what extent molecular methods can be used to make anamorph-teleomorph connections in the Xylariaceae in the absence of a teleomorph is unknown. Recent examination of ribosomal DNA restriction patterns in single conidial cultures of *X. magnoliae* J.D. Rogers derived from stromata from single host fruits, from different host fruits and from different host tree localities indicate considerable variability in ribosomal DNA phenotypes within localities and from single fruits (Gowan and Vilgalys 1991). Therefore the amount of molecular data required before unequivocal identification of a particular xylariaceous taxon can be achieved under a range of different situations is an unknown quantity but is likely to be more extensive than originally expected.
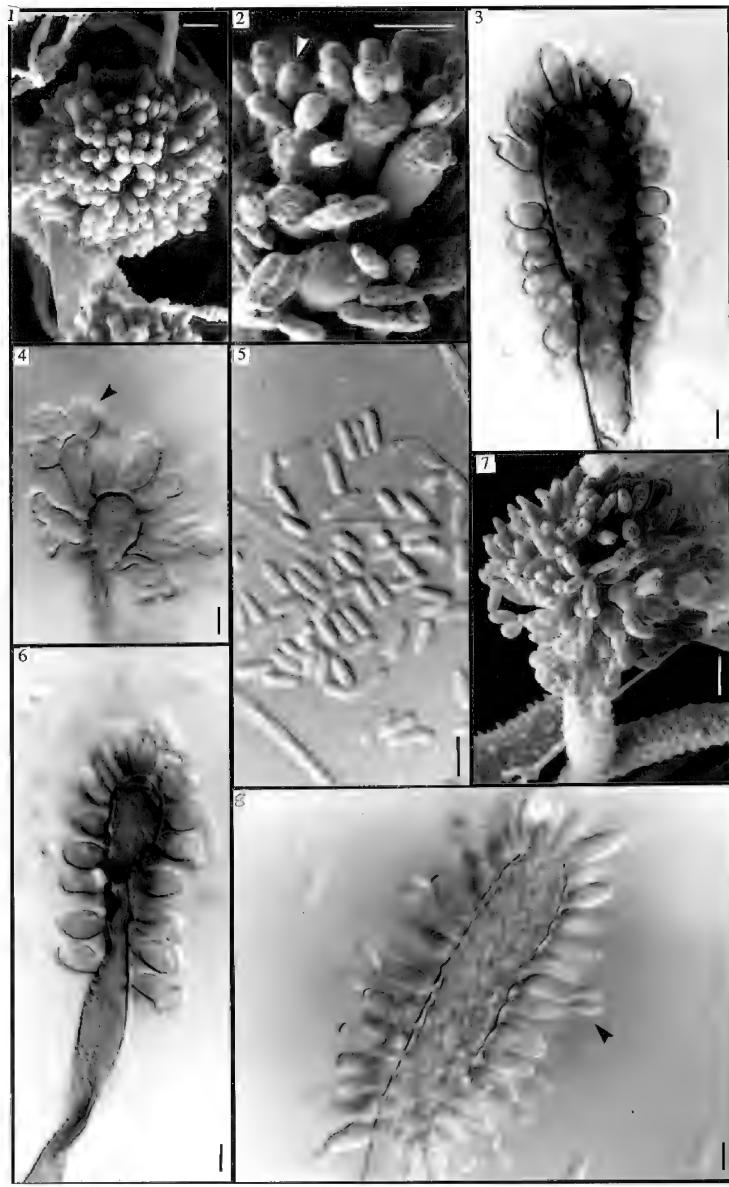
Figs 1-8. *Xylocladium* anamorph of *Camillea leprieurii* (Mont.) Mont. and *C. tinctor* (Berk.) Laessoe, Rogers & Whalley. Scale bar = 5 um. Figs. 1-3. *C. leprieurii* (applanate form). Figs. 1-2. Ampullae, conidiogenous cells and conidia (arrowed). Fig. 3. Ampullae with conidiogenous cells. Figs. 4-6. *C. leprieurii* (erect form). Fig. 4. Formation of secondary conidiogenous cells (arrowed). Fig. 5. Conidia. Fig. 6. Ampullae bearing conidiogenous cells with scars from conidial production. Figs 7-8. *C. tinctor*. Fig. 7. Ampullae with numerous conidia. Fig. 8. Ampullae with conidiogenous cells and conidia (arrowed). Figs 1, 2, 7. Cryo SEM micrographs. Figs 3-6, 8. Differential interference contrast microscopy.

Regardless of the form genera to which Xylariaceous anamorphs are assigned conidiogenesis has been described as holoblastic coupled with a more or less sympodial proliferation of the conidiogenous cell (eg. Greenhalgh and Chesters 1968, Jong and Rogers 1972, Petrini and Muller 1986). The conidia which are produced singly characteristically possess a flattened base relating to their former point of attachment to the conidiogenous cell. Corresponding succession scars on the conidiogenous cell mark the production of single conidia. Since the taxonomic value of the anamorphic state in the Xylariaceae was first recognised by Chesters and Greenhalgh (1964) many xylariaceous taxa have been cultured from ascospores and their anamorphs described and assigned to particular form genera (e.g. Martin 1967, Jong and Rogers 1972, Petrini and Muller 1985). Initial attempts to relate anamorph type with taxonomic groupings were apparently not considered particularly successful at that time (Greenhalgh and Chesters 1968) but the subsequent accumulation of additional data and re-organization of some of these taxonomic groupings has resulted in the recognition of stable anamorph-teleomorph patterns in many genera (Rogers 1985, 1993, Petrini and Muller 1986).

The allocation of particular anamorphs to form genera can in many cases be open to personal interpretation but there is overall agreement that certain genera have clear anamorph connections. Thus *Camillea* Fr. possesses a *Xylocladium* Sydow (=*Basidiobotrys* Hohnel) anamorph (Figs 1-8) (Laessoe, Rogers and Whalley 1991) whilst the dung inhabiting genera *Poronia* Willd. and *Podosordaria* Ellis. & Holw. produce a *Lindquistia* Subram. & Chandrashekara state (Fig. 18) (Stiers, Rogers and Russell 1973, Subramanianand Chandrashekara 1977, Rogers and Laessoe 1992). In these three xylariaceous genera the assigned form genera are currently known to exhibit an exclusive association.

However other anamorphic forms, although distinctively xylariaceous, are more widely occurring and can be associated with a range of, all be it, in the main closely related teleomorphic genera. *Nodulisporium* Preuss (Figs 9-14) is the typical anamorphic form in *Hypoxylon* Bull. s. str., *Daldinia* Ces. & De Not., *Biscogniauxia* O. Kuntze, *Rhopalostroma* Hawksw. and several other genera (Martin 1967, Greenhalgh and Chesters 1968, Jong and Rogers 1972, Hawksworth and Whalley 1985). *Geniculosporium* Chesters & Greenhalgh (Figs 15-16) is associated with *Leprieuria* Laessoe, Rogers & Whalley (Samuels and Muller 1980) *Nemania* S.F. Gray, many species of *Rosellinia* Ces. & De Not. (Greenhalgh & Chesters 1968, Martin 1967, Jong and Rogers 1972, Petrini and Muller 1986, Petrini 1992) *Phylacia* Lev. (Rodrigues and Samuels 1989), *Anthostomella* Sacc. (Francis, Minter and Caine 1980) and *Euepixylon* Fuisting (Whalley 1976, Laessoe and Spooner 1994).
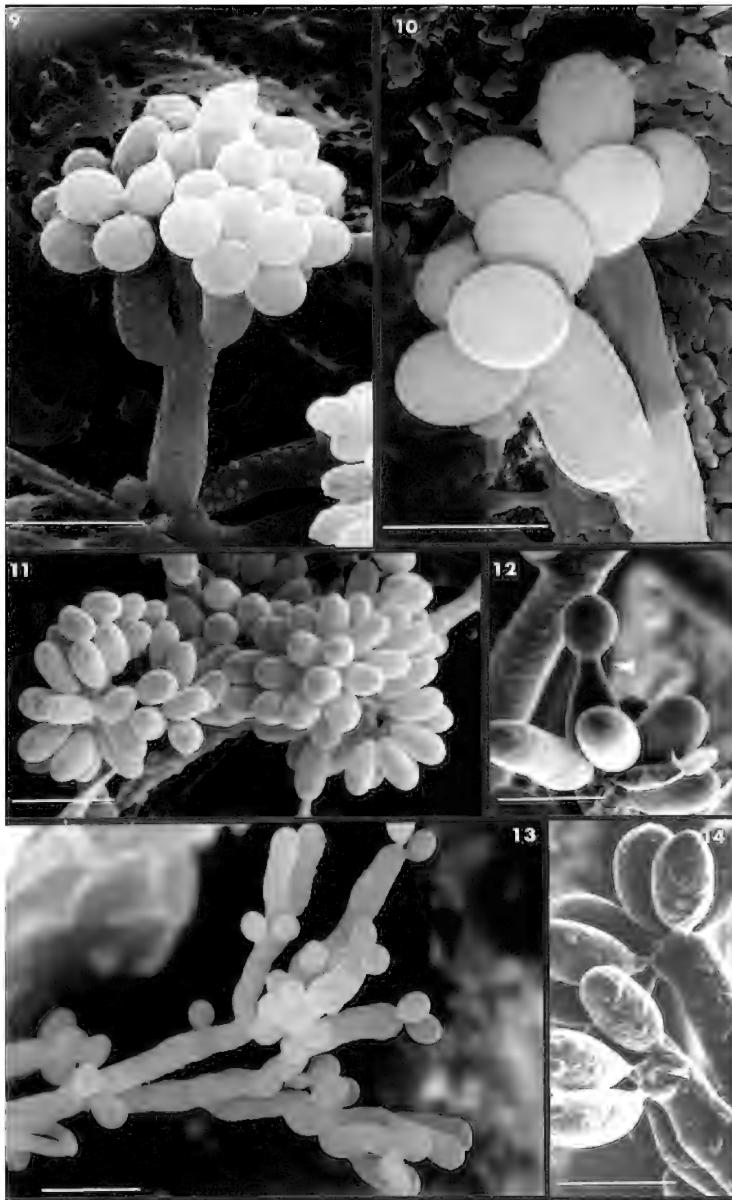
Figs 9-14. *Nodulisporium* anamorph of *Biscogniauxia nummularia* (Bull.:Fr.) O. Kuntze and *Daldinia eschscholtzii* (Ehrenb.) Rehm.
Scale bar = 10 um. Figs 9, 10, 13. *B. nummularia* conidiogenous cells and conidia. Figs. 11, 12, 14. *D. eschscholtzii*. Fig. 12. Young conidium developing (arrowed). Fig. 14. Succession scar on conidiogenous cell marking the former point of attachment of a conidium (arrowed). Figs. 9-14. Cryo SEM micrographs.
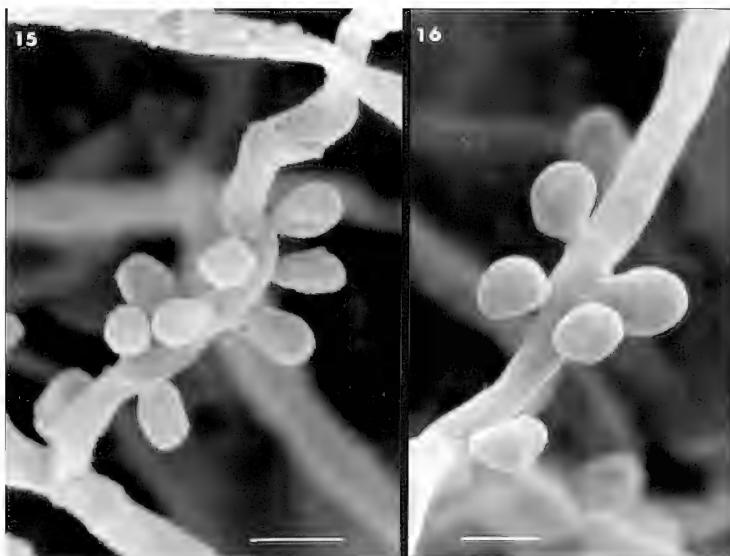

Figs 15-16. *Geniculosporium* anamorph of *Nemania serpens* (Pers.:Fr.) Gray. Scale bar = 10 um. Cryo SEM micrographs.

Geniculosporium Chesters & Greenhalgh (Figs 15-16) is associated with *Leprieuria* Laessoe, Rogers & Whalley (Samuels and Muller 1980) *Nemania* S.F. Gray, many species of *Rosellinia* Ces. & De Not. (Greenhalgh and Chesters 1968, Martin 1967, Jong and Rogers 1972, Petrini and Muller 1986, Petrini 1992) *Phylacia* Lev. (Rodrigues and Samuels 1989), *Anthostomella* Sacc. (Francis, Minterand Caine 1980) and *Euepixylon* Fuisting (Whalley 1976, Laessoe and Spooner 1994). If *Nodulisporium* can be seen as the main anamorphic type in the family it should be noted that there are variations on the theme and various authors have selected *Periconiella* Sacc., *Virgariella* S.J. Hughes or *Rhinocladiella* Nannf. as more appropriate depending on subtlety of branching and degree of complexity of the conidiophores. Thus Petrini and Muller (1986) elected to place anamorphic states of *Biscogniauxia* in *Periconiella* but Gonzalez and Rogers (1992) preferred *Nodulisporium*. Jong and Davies (1974) selected the similar *Sporothrix* Hektoen & C.F. Perkins as the most appropriate form genus to accommodate *Phaeosporis melasperma* (Nyl.) Clem. (=*Areolospora terrophila* S.C. Jong & E.E. Davies.

An interesting and unusual variation is shown by *D. occidentalis* Child where the anamorph, although essentially a *Nodulisporium*, produces its conidia holoblastically where each scar apparently represents the former production site of a single conidium. Thus the conidiogenous cells are annellated and do not exhibit the usual sympodial proliferation typical for a *Nodulisporium* (Petrini and Muller, 1996). A similar observation has been made for *X. longipes* Nits. (Rogers 1983). To what extent these variations are significant in relation to systematic arrangements in the Xylariaceae is unknown but it should be noted that even the distinction between *Nodulisporium* and *Geniculosporium* has been questioned (Cole 1971, Kendrick and Carmichael 1973). However the clear association of *Geniculosporium* with *Nemania, Rosellinia* and a number of other closely related genera provides powerful reason for maintaining separate status for these two genera. The *Dematophora* Hart. state associated with *Rosellinia necatrix* Prill. can be considered as a synematious form of *Geniculosporium* and therefore still in harmony with the anamorphs produced by most other species of *Rosellinia* (Teixeira de Sousa and Whalley 1991).

However a fundamental question raised by Cole (1973) which still requires attention concerns his comment that "it is often difficult to be sure whether a conidiophore or conidiogenous cell has proliferated sympodially when only the mature state is examined". According to Cole " in *Nodulisporium hinnuleum* (Preuss) G. Smith conidia are produced in a basipetal succession, clustering about the apex of the conidiogenous cell, which does not itself change in length during the process of conidium formation". This is in conflict with the usual interpretation of *Nodulisporium* anamorphs in the Xylariaceae where the conidia are produced by continuous acropetal succession with the youngest conidium produced at the apices of a succession of growing points produced by a sympodially proliferating conidiogenous cell (Greenhalgh and Chesters 1968).

Although the overall pattern of anamorphic type in the Xylariaceae can be viewed as one of consistency there are some notable exceptions. As Ju, Gonzalez and Rogers (1993) stated "one of the hallmarks of taxa of family Xylariaceae is the type of hyphomycetous anamorph that features dry amerosporous conidia produced holoblastically from sympodially proliferating conidiogenous cells". Their discovery of three Xylariaceous taxa [*Lopadostoma turgidum* (Pers.:Fr.) Traverso; *Creosphaeria sassafras* (Schwein.:Fr.) Ju, Gonzalez & J.D. Rogers and a fungus close to *Nummularia viridis* Theiss.] that, "in culture, produce more or less slimy scolecosporous conidia holoblastically from sympodially proliferating or sympodially and percurrently proliferating conidiogenous cells" (Ju *et al*. 1993) is an interesting development. It leads them to conclude "that these fungi represent a heretofore unrecognized evolutionary line in the Xylariaceae" ... and... "the relationships among these three taxa are presently unknown" (Ju *et al*. 1993). Although these undoubtedly represent a major departure from the 'norm' for the family there are other curiosities. *Padixonia* C.V. Subraman. (Fig. 20) is unique to *Xylaria furcata* Fr., one of several species associated with termite nests (Subramanian 1972) and *Acanthodochium* Samuels, Rogers and Nagasawa (Fig. 19) has to date been reserved for anamorphs of *Collodiscula japonica* Hino & Katumoto and *Astrocystis* Berk. & Broome (Samuels, Rogers and Nagasawa 1987, Ju and Rogers 1990).
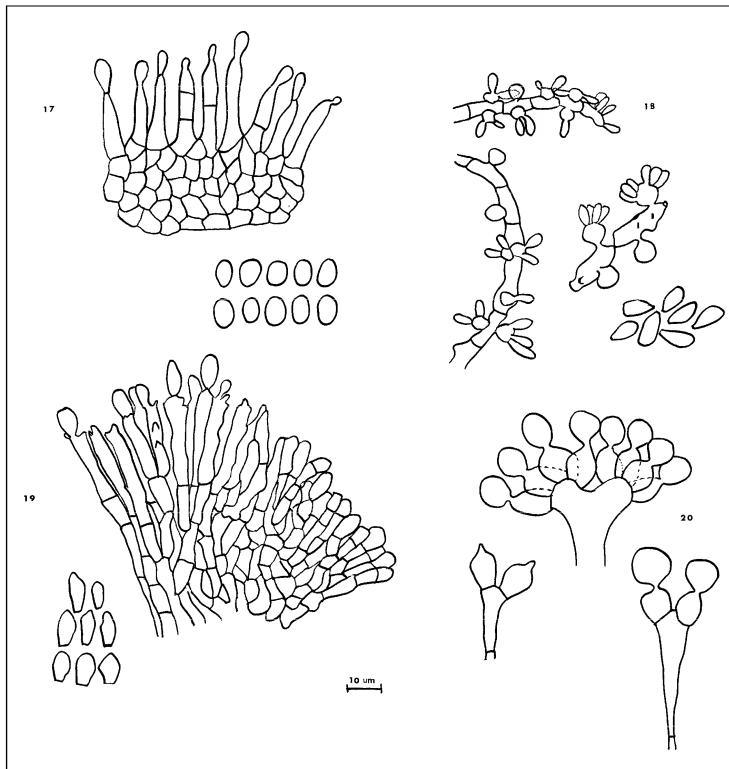
Figs 17-20. Conidiogenous cells and conidia of Fig.17. *Hadrotrichum* (after Petrini and Candoussau), Fig. 18. *Lindquistia* (after Subramanian and Chandrashekara), Fig. 19. *Acanthodochium* (after Samuels *et al.*), Fig. 20. *Padixonia* (after Subramanian).

Other isolated anamorphic forms which deserve consideration include *Hadrotrichum* Fuckel which was the chosen form genus for the anamorph of *Hypoxylon deustum* (Hoffm.:Fr.) Grev. (= *Kretzschmaria deusta* (Hoffm.:Fr.) P.M.D. Martin (Petrini & Muller, 1986) and the *Xylocoremium flabelliforme* (Schwein.:Fr.) J.D. Rogers anamorph of *Xylaria cubensis* (Mont.) Fr., *X. allantoidea* (Berk.) Fr. and allies (Callan and Rogers 1990). We draw attention to *Hadrotrichum pyrenaicum* Petrini & Candoussau and the statement that "*Hadrotrichum pyrenaicum* has probably been overlooked, or confused, for a long time with immature forms of *Hypoxylon* spp., although a microscopical examination leaves no doubt as to its identity. No connection with any known species of *Hypoxylon* could be established even though the presence of *Nodulisporium* - like conidiophores and conidia in pure culture indicate a possible link" (Petrini and Candoussau 1983). Later Petrini and Muller (1986) connected *H. pyrenaicum* with *Hypoxylon*.

moravicum Pouzar. It is interesting to note that *Hypoxylon fraxinophillum* Pouzar (=*H. argillaceum* Berk.) is also restricted to *Fraxinus* (Pouzar 1972) and it develops conidiophores, which as illustrated by Greenhalgh & Chesters (1968), are remarkably similar to those of *H. pyrenaicum*. It certainly would not be unexpected to find similar anamorphs in these closely related taxa but the presence of a *Hadrotrichum* anamorph in *Hypoxylon deustum* as recorded by Petrini and Muller (1986) is much more difficult to explain in terms of teleomorph relationships. It is generally agreed that *Hypoxylon deustum* has little in common with *Hypoxylon* s. str. and it is usually referred to *Ustulina* Tul. & C. Tul. or to *Kretzschmaria* Fr. if the proposal to synonymize these genera is accepted (Laessoe, 1994).

   Regardless of argument over the finer details of assignment to hyphomycetous genera the value of the anamorph in developing systematic arrangement within the Xylariaceae must not be undervalued. As stated by Rodrigues and Samuels (1989) in reference to *Phylacia* "Anamorphs can be useful in determining relationships of fungi" and "the anamorphs that we observed are species of *Geniculosporium* (sensu Petrini and Petrini 1985) and are wholly consistent with anamorphs that have previously been described for the family Xylariaceae". In a similar vein Callan & Rogers (1990) stated that "*Xylaria* Hill ex Schrank is increasingly recognized as a diverse and complicated assemblage of taxa. It has become apparent that the biology and systematics of *Xylaria* and its allies can be understood only after consideration of the holomorphs".

Table 1. Teleomorph-anamorph connections in the Xylariaceae

TELEOMORPH	ANAMORPHS
Anthostomella Sacc.	*Nodulisporium & Virgariella*
Astrocystis Berk. & Broome	*Acanthodochium*
Biscogniauxia Kuntze	*Geniculosporium, Periconiella &*
	Nodulisporium
Calceomyces Udagawa & Ueda	*Nodulisporium*
Camillea Fr.	*Xylocladium*
Collodiscula * I.Hino & Katum.	*Acanthodochium*
Daldinia Ces. & De Not.	*Nodulisporium*
Entonaema A.Moller	*Nodulisporium*
Euepixylon * Fuisting	*Geniculosporium*
Hypoxylon Bull.	*Nodulisporium, Virgariella*
	&Rhinocladiella
Induratia Samuels, E.Mull. & O.Petrini	*Nodulisporium*
Leprieuria Laessoe, J.D.Rogers & Whalley.	*Geniculosporium*
Nemania * S.F.Gray	*Geniculosporium*
Obolarina * Pouzar	*? Rhinocladiella*
Phaeosporis Clem.	*Sporothrix*
Phylacia Lev.	*Geniculosporium*
Podosordaria Ellis & Holw.	*Lindquistia*
Poronia Willd.	*Lindquistia*
Rhopalostroma D.Hawks.	*Nodulisporium*
Rosellinia De Not.	*Geniculosporium, Dematophora*
	& Nodulisporium
Thamnomyces Ehrenb.	*Nodulisporium*
Theumenella Penz. & Sacc.	*Nodulisporium*
Xylaria Hill ex Schrank	*Xylocoremium*
* not recognised by Eriksson & Hawksworth (1993)	.

   The value of cultural studies in the Xylariaceae is clearly proven: at their least the anamorphs provide a range of new characters for taxonomic purposes but their

outstanding contribution lies in the confirmation of connections with known teleomorphs and the subsequent development of more natural groupings. Currently accepted anamorph teleomorph connections in the Xylariaceae are listed in Table 1; others will undoubtedly follow.

Acknowlegments
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ANAMORPHS AND THE CLASSIFICATION OF XYLARIACEOUS FUNGI

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Introduction

As Rogers (1985) pointed out *Xylaria* Hill ex Schrank is a complex and difficult genus. Several mycologists (Fries 1851, Miller 1942, Dennis 1961, 1970, 1974, Joly 1968, Martin 1970, Bertault 1984) have tried to organize the genus into well-defined groups, based upon stromatal characteristics. Though these efforts have some merit, most of the proposed schemes are difficult to use consistently and the groupings tend to be artificial due to variability and often ambiguous nature of some of the major characters employed.

Classifiction

Rogers (1985) was the first to propose a subdivision of the genus *Xylaria* based upon anamorphic characteristics. The division is primarily based on two aspects of *Xylaria* anamorphs: the place of the anamorph in the life cycle, and the morphology of the conidiogenous structure. He recognizes 8 groups divided upon 4 sections (see table 1).

Table 1. The subdivision of *Xylaria* into four sections based on anamorph characteristics (Rogers 1985).

sections	characteristic features
Section 1	Conidia produced from palisade of conidiogenous cells over entire young telemorphic stromata or at least not limited to specialized appendages. Conidiogenesis holoblastic. Conidia produced in more or less sympodial sequence, seceding individually and passively. This section contains 5 groups: *Xylaria polymorpha* group, *X. hypoxylon* group, *X. multiplex* group, *X. pyramidata* group and the *X. pedunculata* group.
Section 2	Conidia produced on special and localized peg- or hair-shaped appendages on young teleomorphic stromata. Conidiogenesis apparently holoblastic. Conidia apparently seceding individually and passively. This section is represented by the *X. comosa* group.
Section 3	Conidia produced on special anamorphic stromata or conidiomata which usually are produced earlier in the year than teleomorphic stromata. Teleomorphic stromata never bear conidia. Conidiogenesis holoblastic. Conidia produced in more or less sympodial sequence, seceding individually and passively. This section is represented by the *X. cubensis* group.
Section 4	Conidia produced on young teleomorphic stromata. Conidiogenesis apparently holoblastic. Conidia produced in tandem, seceding forcibly. This section is represented by the *X. furcata* group.

The division proposed by Rogers (1985) has special merit, since the recognized sections may represent more natural assemblages of *Xylaria* species. However, as the author pointed out many more anamorphic data must be obtained and correlated with teleomorphic data to fill in gaps and thereby ameliorate and/or alter the proposed system.

The subdivision proposed here, which became apparent through my study of anamorphic and teleomorphic features of Papua New Guinean material, is inspired

on the work of Rogers (1985), but differs in the grouping of some of the species. These differences can largely be explained by the fact that I have been able to study the anamorphic state (including the anamorph as found in nature) of a number of species for which there were no previous data.

Based on the Papua New Guinean material 5 groups could be recognized.

1. *Xylaria anisopleura* group

Members of this group are *X. anisopleura* (Mont.) Fr., *X. schweinitzii* Berk. & M.A. Curtis, and, judging from the teleomorphic features, *X. scruposa* (Fr.) Fr.

- *Teleomorphic features*: This group is well characterised by the (usually) large teleomorphic stromata with a roughened stromatal surface due to wrinkles, warts and tomentum, with greyish to brownish scales when young, becoming blackish to black at maturity; ostioles umbilicate, appearing as small hemispherical black discs; apical apparatus rectangular, constricted sub-apically; ascospores fairly large, over 15 µm long, usually with a short, straight to curving germ slit, obliquely oriented.

- A*namorph in nature*: The anamorph as found in nature is formed on stromata growing side by side with mature teleomorphic stromata. These stromata are, except for being smaller, similar to the teleomorphic stromata (e.g. clavate to spathulate for *X. anisopleura* (Mont.) Fr., and cylindric-fusoid for *X. schweinitzii* Berk. & M.A. Curtis).

Most probably, the anamorph in this group develops on young immature teleomorphic stromata, iIn contrast to the taxa of the *X. cubensis* group (see further) where the anamorph develops on stromata distinct from the teleomorphic stromata.

- *Cultural characteristics*: The colony colours are white, grey and black, and the mycelium is characterised by the presence of coiled hyphae. The conidia are produced upon cylindrical stromata and are ellipsoid. In most instances, the cultures remained sterile, or the material did not germinate at all.

This group represents part of the *X. polymorpha* group as recognized by Rogers (1985) (see further).

2. *Xylaria feejeensis* group

Members of this group are *X. feejeensis* (Berk.) Fr. and *X. luteostromata* Lloyd.

- *Teleomorphic features*: Characteristic for this group are the slender stromata, usually less than 5 mm diam., with a surface finely and reticulately cracked into small, angular closely spaced scales so as to outline the individual perithecia; ostioles finely papillate in the centre of a dome-shaped to hemispherical black disc; apical apparatus more or less quadrate; ascospores rather small (up to 11 µm long) with a straight germ slit running almost full spore length.

- *Anamorph in nature*: unknown.

- *Cultural characteristics*: Colonies appressed, white to grey (usually with a reddish to orange shine). The mycelium is characterised by the presence of highly coiled hyphae. Conidia are produced upon cylindrical stromata or on small rudimentary stromata. Conidia subglobose, obovoid to cylindrical.

Rogers (1985) placed *X. feejeensis* (Berk.) Fr. together with *X. anisopleura* (Mont.) Fr. and *X. schweinitzii* Berk. & M.A. Curtis in the *X. polymorpha* group. Though cultural characteristics indicate that the *X. feejeensis* group and the *X. anisopleura* group may be closely related, it is my opinion that based on the above mentioned differences in teleomorphic and anamorphic features warrent their classification in two distinct groups.

3. *Xylaria apiculata* group

Members of this group are *X apiculata* Cooke, *X. palmicola* G. Winter and *X. mellisii* (Berk.) Cooke.

- *Teleomorphic features*: Stromata with cylindric-conical fertile parts, on narrow hirsute stipes, and attenuated acute sterile apices; surface with peeling or flaking greyish to brownish outer layer, the remnants persisting as conspicuous plates or shreds in some species or not noticeably persisting in other species; ostioles umbilicate, usually obscure, or slightly raised; ascospores over 10 µm long with a straight or slightly spiralling germ slit, from less than to almost full spore length.

- *Anamorph in nature*: formed on filiform apiculi or branches, white to grey, of immature teleomorphic stromata. Conidia obovoid, small, 3.5-5.5x1-2.5 µm.

- *Cultural characteristics* (after Rogers and Samuels 1986, Rogers *et al.* 1988).
Colonies velvety, white, becoming grey to black (with a greenish shine). Slender cylindrical stromata are usually formed, less than 2 mm diam., covered with dark hyphae and with an acute white tip. Conidia rarely forming in culture, arising from loosely dispersed, aerial hyphae along the white upper part of the stroma.

Rogers (1985) recognized a *X. multiplex* group with the following members: *X. apiculata* Cooke, *X. arbuscula* Sacc. (= *X. mellisii* (Berk.) Cooke), *X. multiplex* (Kunze) Fr. and *X. schreuderiana* van der Bijl. I agree on the grouping of *X. apiculata* Cooke and *X. arbuscula* Sacc. but according to my observations, *X. multiplex* (Kunze) Fr. belongs to a group clearly distinct from the here proposed *X. apiculata* group (see further).

4. *Xylaria grammica* group

Members of this group are *X. grammica* (Mont.) Fr., *X. papulis* Lloyd, *X. multiplex* (Kunze) Fr. and *X.* cf. *pallida* Berk. & Cooke.

- *Teleomorphic features*: Immature stromata brightly coloured, white or yellow. The brightly coloured surface layer is worn off in mature stromata. Surface of mature stromata smooth, with remnants of the bright-coloured surface layer, or characterized by an outer layer which splits longitudinally around the ostioles, exposing the underlying black surface. Ostioles punctiform and located in the black stripes, or finely papillate, often surrounded by an annular disc; ascospores inaequilaterally ellipsoid with a straight germ slit running almost full spore length.

- *Anamorph in nature*: formed on wart-like structures developed upon immature bright coloured teleomorphic stromata.

- *Cultural characteristics*: Colonies at first brightly coloured (e.g. light yellow), later darkening from centre outwards, finally becoming black with a greenish shine. Mycelium characterized by the presence of thick walled, dark brown hyphae, with short protuberances, breaking down into segments. Cylindrical stromata covered with villose black hyphae are formed, but they usually remain sterile, or the conidiogenous structures are produced on wart-like structures upon the cylindrical stromata. Anamorph usually formed on small tufts or subglobose stromata in the centre of the colony. Conidia elongated ellipsoid.

This is a conspicuous group due to the bright colouration of the colonies as well as of the immature stromata, the production of conidia on wart-like structures, and the shape of the conidia. This group was not previously recognized.

5. *Xylaria cubensis* **group**

Members of this group are *X. cubensis* (Mont.) Fr., *X. allantoidea* (Berk.) Fr. and *X. poitei* (Lév.) Fr. This group corresponds to the *X. cubensis* group as described by Rogers (1985).

- *Teleomorphic features*: Stromata brown, bronze or copper-coloured, becoming blackened with age; surface smooth and plane; ostioles papillate; ascospores inaequilaterally ellipsoid, germ slit inconspicuous or conspicuous, straight, running almost full spore length.

- *Anamorph in nature*: produced on special anamorphic stromata which develop earlier in the year than the teleomorphic stromata.

- *Cultural features*: Colonies orange-white. Anamorph formed on cylindrical to flabelliform stromata. Conidiogenous cells long cylindrical. Conidia obovoid.

Conclusion

It was not possible to place all the *Xylaria* taxa, found in Papua New Guinea, in one of the above circumscribed groups, especially due to the lack of observations on the anamorphic state.

It is obvious that much more study needs to be done before one can come to a more natural subdivision within the genus *Xylaria*. In my opinion, however, the subdivision as proposed by Rogers (1985) together with the scheme presented above, though both far for complete and in need of further corroboration, provide an indication that the combination of anamorphic and teleomorphic features can lead to a less artificial grouping, and may thus provide insight into the evolution of the large and complex genus *Xylaria*.

It is also admitted that a subdivision of a complex genus like *Xylaria* should ideally be carried out in the frame-work of a worldwide revision rather than being based on a study of the funga[3] of one region. However, I have judged it worthwhile to propose the scheme obtained, such that it can be verified and complemented through studies on taxa from other regions.

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First occurrence in nature of the teleomorph of *Cochliobolus*

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Introduction

The genus *Cochliobolus* was erected by Drechsler (1934) to include those fungi with teleomorphs producing asci with helically coiled filiform ascospores. Teleomorphs of *Cochliobolus* are rare in nature (Sivanesan 1987). The teleomorph *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoem., syn. *Helminthosporium sativum* Pammel, King & Bakke) syn. *Drechslera sorokiniana* (Sacc.) Subram. & Jain, has not been recorded in the field (Sivanesan 1987, personal comm. 1990, Tinline and Dickson 1958).

Sivanesan (1987) reports that several *Cochliobolus* spp. were produced since 1927 by pairing opposite mating strains of single spore isolates at temperatures above 20 °C on agar media prepared with plant material. *Cochliobolus sativus* was produced in the laboratory and described by Ito and Kuribayashi, and by Tinline (1951) and Tinlin and Dickson (1958).

This paper describes the occurrence in nature of *C. sativus* in Zambia following the introduction of rainfed wheat, on which annually epidemics of *Bipolaris sorokiniana* diseases occur (Raemaekers 1991, Raemaekers *et al.* 1991). The naturally occurring teleomorph is compared with the teleomorph produced by Ito & Kuribayashi and by Tinline (1951) in the laboratory.

Materials and methods

Rainfed wheat or summer wheat research was re-introduced by the Department of Agriculture (Zambia) in the late 1970s. The research described was carried out at Mount Makulu Research Station (MMRS) near Lusaka, where wheat plots were seeded early January and harvested in May. At this and at other locations in the country, *B. sorokiniana* diseases (spot blotch, head blight, stem break and black point) were destructive (Raemaekers 1991).

The weather conditions during the period from harvest to seeding of the new crop (autumn to summer) when the teleomorph develops are summarised (Table 1).

To examine the above-ground crop residues from autumn till summer, wheat culms with and without ears were collected after plot-combining and taken to an area (garden) protected from bushfires. There, the straw was spread on a net of chickenwire (1 x 8m) suspended ± 20cm above soil level in an area without shade. It was exposed to normal weather conditions, similar to those occurring in the adjacent fields. This procedure started in 1985 but due to circumstances it had to be repeated annually till 1989-90. Straw from the Mbala area, 1,000km north of Lusaka, was collected after harvest and exposed to the weather in the same way. Partially decomposed straw, used as mulch under coffee trees in the same area, was also collected.

Selected parts were examined at regular intervals by stereoscope and fruiting bodies were carefully removed, often from in between a dense mat of conidia and conidiophores. They were cleaned following a method described by Tinline (1951). A cleaned fruiting body would then be mounted in a drop of sterile distilled water and squashed for microscopic examination.

Table 1. Weather conditions from autumn to summer at MMRS[1]

month	mean temp. °C	mean max temp. °C	mean min temp. °C	% R.H.	Sunshine hrs/day	rainfall mm
May	17.3	24.5	11.3	57	9.1	5
June	16.1	23.3	9.8	55	8.5	0
July	16.0	23.2	9.2	51	9.0	0
August	18.3	25.7	11.9	44	9.1	0
September	22.0	29.4	16.4	37	9.5	1
October	24.8	31.8	18.4	40	8.8	15
November	22.7	29.0	17.9	59	6.6	97
December	21.1	26.3	17.2	74	5.7	222
January	21.0	26.3	17.4	75	4.9	166

[1]MMRS: Mount Makulu Central Agricultural Research Station, Chilanga, Zambia. (Meteorological Department, Lusaka, 1975).

Asci and single spores were placed on PDA with bactericide. Single spores were separated under the stereoscope into a small drop of sterile distilled water and transferred to the agar. Petri dishes were sealed and kept at room temperature in the laboratory where they were exposed to daylight.

Plants of the susceptible wheat variety 'Loerie' were grown in pots in the greenhouse for pathogenicity tests. At heading, the bottom leaves were removed and the two top leaves were kept for inoculation. In the laboratory, these plants were atomised with distilled water. A drop of sterile distilled water was placed a few cm away from the leaf base either on the flag leaf or the penultimate leaf. The following treatments were compared with a control: single *B. sorokiniana* conidium from wheat straw, single conidium from monoascosporic isolate, single ascospore, groups of ascospores, ascus. Spores were collected with fine pointed tweezers under the stereoscope and transferred to the drop of water on the leaf. The plants were covered with plastic bags after being atomised with distilled water. The pots were placed on a bench in a tray with water. The bags were removed 24 hours later. The maximum and minimum temperatures in the laboratory were 29 and 22°C respectively during the period 15 to 30 March 1990.

Results

Ascocarps developed in abundance towards the end of the year on the straw; this occurred annually during the years of observations. Most ascocarps were observed on dark brown discoloured straw and ears, the discolouration being the result of severe infection during crop life. Conidia and conidiophores were also present in abundance on this dry material. The ascigerous stage was present predominantly on the upper part of the plant including glumes, rachis, peduncle, top internode and nodes, but not on the lower internodes. It was not observed on the infected grains in the glumes. Most of the leaves were disintegrated by the time the ascocarps started to develop; fruiting bodies did develop in and underneath leaf sheaths.

Mature fruiting bodies started to develop in December. By March-April, most ostioles were open and such ascocarps were found to be empty upon examination. A few immature (nonseptate) spores were observed by mid-December. By late December the first septate ascospores were noticed. The first observation of such a spore was on 24.12.85. By mid-January, pseudothecia with immature and mature asci were present. One hundred and four fruiting bodies were examined mid-

January 1986. Only 15% of those contained asci (with mature and immature ascospores). In February and March 1990, only one third of all asci examined in many fruiting bodies contained ascospores. The other asci were either empty or filled to various degrees with granular protoplasma. Pseudothecia always contained a mixture of asci with and without ascospores. Ascocarps developed on the Mbala straw, but less frequent than on the more infected MMRS straw. A few ascocarps with mature ascospores were found on the partially decomposed straw (mulch).

Globose superficial pseudothecia were smooth and black, occasionally with setae. Most of them developed amidst concentrations of conidia and conidiophores from the infection during crop growth. Often they were held to the straw by strips of disintegrating epidermis through which they had grown. They were usually scattered on the wheat tissue. Occasionally they developed closely together in groups of 4 to 6. Most ascocarps had a short subcylindrical ostiolar beak. Several pseudothecia were observed without beak; one fruiting body had two beaks. Upon contact with water on a glass slide, or after being crushed by a needle if not opening spontaneously, the mature fruiting bodies released their content of asci and paraphyses together. Asci in all stages of development appeared.

The number of asci per ascocarp varied between 1 and 39 with a mean of 16. One ascocarp with 50 asci was observed. The number of asci with spores varied between 1 and 9 per ascocarp. The number of ascospores per ascus varied from 1 to 8, with a mean of 5. Similar to descriptions by Drechsler (1934) and Tinline (1951), the shape of the asci was subcylindrical to clavate, slightly curved and short-stipitate.

When the ascus was filled with spores, they were always coiled in a tight helix, the typical characteristic of the genus *Cochliobolus*. Coiled spores discharged simultaneously through the ascus apex which ruptured circumscissally. The ascospores uncoiled gradually in water. Asci contained any number of spores from one to eight. When an ascus contained one ascospore only, this one was bent or positioned irregularly within the ascus. Nonseptate spores were hyaline; mature spores somewhat olivaceous. They were long, filiform and with many septa. Constrictions at the septa were visible, especially at or prior to germination. Mature ascospores broke up easily into segments which germinated individually.

Single septated ascospores quickly germinated on PDA and after 2 to 3 hours the developing germtube was visible. Nonseptate ascospores did not germinate. Spores not liberated from within the ascus germinated *in situ* through the ascus wall on PDA.

Table 2. Comparison of measurements of *C. sativus* naturally occurring *versus* laboratory produced teleomorph (units in µm)

	C. sativus in nature		*C. sativus* in laboratory[1]	
		mean	Tinline 1951	Ito
ascocarp width	240 - 660	(440)[2]	310 - 460	370 - 530
ascocarp height	180 - 576	(383)	270 - 420	340 - 470
beak width	50 - 144	(85)	90 - 140	80 - 110
beak length	40 - 90	(69)	66 - 190	90 - 150
ascus length	43 - 259	(162)	160 - 255	110 - 220
ascus width	10 - 39	(27)	27 - 42	32 - 45
ascospore length	113 - 420	(220)	182 - 343	160 - 360
ascospore width	7 - 15	(9)	5 - 10	6 - 9
number of septa	3 - 11	(7)	6 - 14	6 - 13

Table 2 compares the measurements of ascocarps, asci and ascospores of *C. sativus* occurring in nature with those recorded by Ito and Kurib (Tinline 1951) of artificially produced teleomorphs made by mating compatible fertile strains under controlled laboratory conditions.

In general, the observations show the species to have a wider range of measurements than previously recorded. Some ascocarps found in nature were smaller while others were larger than those produced in the laboratory. Longer beaks were recorded on the laboratory produced teleomorphs. The ascus measurements include both mature and immature asci. Immature asci varied in length between 50 and 250 μm and in width between 10 and 38 μm. The length of mature asci fluctuated between 43 and 246 μm. In the range of 50 to 110 μm only immature asci were observed. Most ascus lengths fell within the range covered by Tinline's and Ito's, but the asci were somewhat narrower than theirs. Most measurements of ascospore length and width were within the range observed by both authors. Some were wider and shorter; longer ascospores were observed as well. Nonseptate ascospores measured 134-276 μm by 5-10 μm. The maxima of 14 and 13 septa as observed by Tinline and Ito & Kuribayashi respectively were not observed.

Monoascosporic cultures yielded *Bipolaris sorokiniana* with typical brown conidia and dark mycelium on PDA, identical to cultures from single conidia directly transferred from straw to the agar medium.

In the pathogenicity tests, all inoculations produced tiny brown specks within 48 hours. Within 4 days such a speck developed into a light brown elliptical lesion with a dark centre and a yellow halo. Six days after inoculations, small parts of the lesions were plated on PDA and *B. sorokiniana* was recovered.

Discussion

This is the first report of abundant formation in nature and annual re-occurrence in spring of the teleomorph of *Cochliobolus sativus*. A reference sample was deposited in 1990 at the International Mycological Institute under IMI-number 34112 and the fungus was confirmed as *C. sativus* (Sivanesan, personal communication). Moist chamber tests and plating of infected plant parts on agar media never yielded the teleomorph during the period 1974-90.

Tinline (1951) considered the slight differences between his measurements and those by Ito and Kurib. to be due to variant strains of the fungus, and environmental and cultural differences. The differences observed here between the laboratory produced and naturally occurring *C. sativus* may be attributable to different conditions during development in 'controlled environment' in Canada and Japan and 'normal weather conditions' in Zambia.

The occurrence of asci with fewer than 8 ascospores, also observed by others (Drechsler 1934, Nelson 1964, Tinline 1951), is attributed to the chromosomal behaviour in the developing ascus (Hrushovetz 1956). This may also explain why many asci were either empty or partially filled with granular protoplasma, at a time when most asci were expected to contain mature ascospores.

The presence of the perfect stage in central and northern Zambia at locations 1,000km apart and the severity of *B. sorokiniana* infections in rainfed wheat experiments in central Zimbabwe (Raemaekers 1987) are an indication that the teleomorph could develop abundantly in a large region of southern Africa, if a susceptible crop such as summer wheat is introduced.

Tinline and Dickson (1958) reported that the formation and maturation of *C.*

sativus was greatly affected by temperature, requiring more precise environmental conditions than the vegetative stage. Sexually the fungus developed only on natural media between 16 and 24°C with the optimum at 20°C, whereas asexually it grew on natural and synthetic media between 4 and 36°C with optimum growth at 28°C. Shoemaker (1955) investigated the temperature range and observed that the formation of male and female reproductive organs in *C. sativus* took place at 24 and 20°C respectively. Beyond these temperatures no fertilisation took place and only protothecia developed. Similar temperatures as those at which the teleomorph was produced in the laboratory occur in Zambia during spring and early summer.

C. sativus and other *Cochliobolus* species are heterothallic (Nelson 1957, Tinline 1951). Opposite mating strains are randomly distributed in nature, and may even occur on the same lesion (Nelson 1957). The dense concentration of *Bipolaris sorokiniana* on wheat straw in Zambia therefore is thought to contain both mating strains.

What the implications are of finding the complete life cycle of *Cochliobolus sativus* in nature in Zambia is not yet clear. It was demonstrated that it can serve as primary inoculum along with conidia from debris. Its role will be better understood when rainfed wheat production expands and wheat-wheat rotations are introduced. Production of more virulent *C. sativus* strains is not excluded.

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PYXIDIOPHORA, A POSSIBLE TELEOMORPH OF *PLEUROCATERA ACICULARIS*

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Abstract. In old cultures of the recently re-discovered hyphomycete *Pleurocatena acicularis* mature perithecia were found which doubtless are the teleomorph of this fungus and belongs to the genus *Pyxidiophora*. Until now a connection between *Pyxidiophora* and *Pleurocatena* was unknown.

Introduction

Establishing connections between anamorphs and teleomorphs, or to find a teleomorph of an existing anamorph is one of the tasks even of the modern mycology. There are various methods and techniques to gain positive results, as was pointed out by Kendrick (1979). The factor of a lucky chance should not be forgotten. It was indeed one lucky chance which led us to a surprising possible connection. This "possible" should be underlined, because the result until now was impossible to repeat. The fact is that in our laboratory the teleomorph of *Pleurocaten*a *acicularis* was discovered.

Material and Methods

A pure culture of the hyphomycete *Pleurocatena acicularis* was obtained from conidia from the natural host and subcultured.

The fungus was isolated from a minute colony on *Hymenochaete tabacina*, covered partially with *Hypocreopsis lichenoide*s, collected by Björn Brandt on *Salix aurita*, Germany, Mecklenburg-Vorpommern, between Bad Sülze and Triebsees, 15.03.1992, and identified by G.R.W. Arnold (A 92/39.2 = MW i 3147). The fungus, surely, is mycophilic.

The growth in liquid Czapek medium is almost zero. Subcultures were obtained on malt-agar (MA), oat-meal-agar (OMA) and synthetic nutrient agar (SNA) after Nirenberg, incubated in the incubator-room at 21 ± 1°C and a relative humidity of 76%. The growth of the fungus was checked at great intervals (4-6 weeks).

Results

P. acicularis proved to be a highly difficult fungus. The mycelial growth was, in general on all three employed media, slow and scanty; the best growth was observed on MA, the poorest on SNA. A thin, whitish superficial mycelium was developed. Conidiation, characteristic for *P. acicularis* (Fig. 1), was satisfaying on MA, not so good on OMA, and rare on SNA. But after a period of four months small darkish points were detected with naked eye, which under the dissecting microscope were identified as ascomata! A contamination of *Pleurocatena* with an ascomycete was to be excluded. Therefore, the observed ascomycete had to be connected with *Pleurocatena acicularis*. The perithecia were scattered over the elder parts of the colony which at this time covered almost completely the surface of the agar slant in a 10cm Petri dish; they were superficial, comparatively simple, slender and elongated piriforme, almost translucent, with a fimbriate neck (Fig. 2). Asci were not seen. The ascospores were very peculiar and characteristic: long guttuliforme, with a broader

apex and an attenuated basal part, thinwalled, hyaline, two-to multicelled. In some cases a dark eye-spot could be observed in the upper part of the ascospore (Fig. 3).
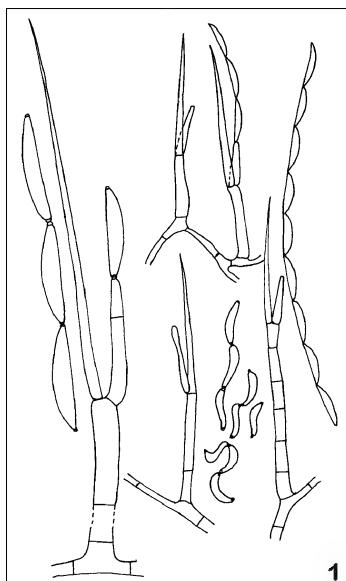
Fig.1. Pleurocatena acicularis: conidiophores and conidia

Until now, the existence of a teleomorph-connection of *Pleurocatena acicularis* was unknown. On the basis of tho described characters, it is proposed to place this ascomycetous fungus in the genus *Pyxidiophora*, tentatively. It certainly differs from the described species of *Pyxidiophora* which have anamorphs belonging to the genera *Chalara* and *Thaxteriola* (Lunquist 1980, Blackwell *et al*.1986).

Conclusions

The formation of a teleomorph of the recently rediscovered and cultivated hyphomycete *Pleurocatena acicularis* (Arnold 1995) was an unexpected surprise. Attempts have to be made to improve the conditions for growth of the fungus, to repeat the production of perithecia based on mono-conidial and mono-ascosporic cultures, to study the physiology of nutrition, biochemistry, a possible antibiotic activity of the fungus, and its ontogeny and cytology.
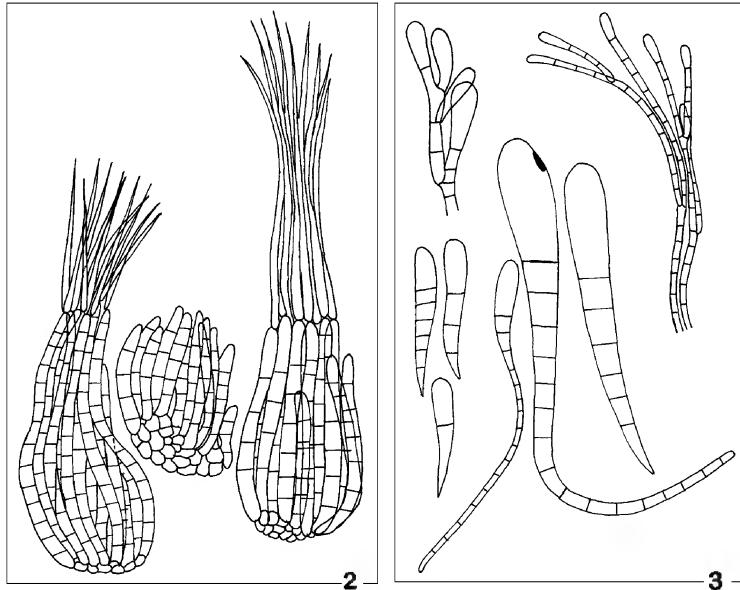
Fig.2. *Pyxidiophora* teleomorph of *Pleurocatena acicularis*: three stages of perithecia

Fig.3. *Pyxidiophora* teleomorph of *Pleurocatena acicularis*: ascospores of different kinds, and"Hullhyphen" of the earlier stages of perithecial development

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ILLUSTRATIONS OF THE CULTURAL FEATURES OF *GYRODONTIUM BOVEANUM* (MONTAGNE) MAAS GEESTERANUS

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Abstract. Main cultural features of *Gyrodontium boveanum* (Montagne) Maas Geesteranus are illustrated on the basis of two cultures which recently became available to the authors. One was obtained from a specimen of *Gyrodontium boveanum* (Montagne) Maas Geesteranus collected by the authors in the small wet forest surrounding the Victoria Falls (Zimbabwe), inside an empty dead trunk. The second was kindly provided by the IFO, Osaka, Japan, under the name *Gyrodontium versicolor* (Berkeley & Broome) Maas Geesteranus.
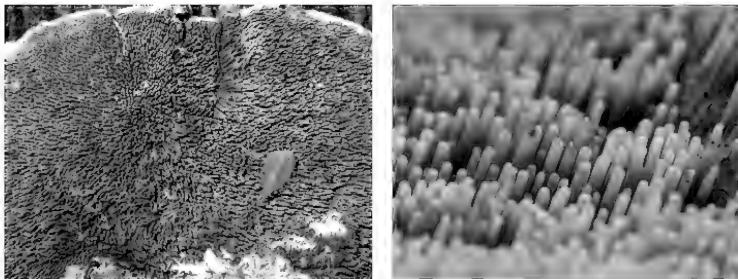
In culture on Malt Agar 2% or on Cherry decoction agar at 25°C, both strains produce arthroconidia. Those arthroconidia rapidly become thick walled, turn yellow brown and round off, soon disjoining and forming a brown powdery layer at the colony surface. Fresh cultures obtained from teeth produced fruitbodies in Petri dishes but, after several subculturing, new fruitbody formation were no more observed. Thin strands are also formed.
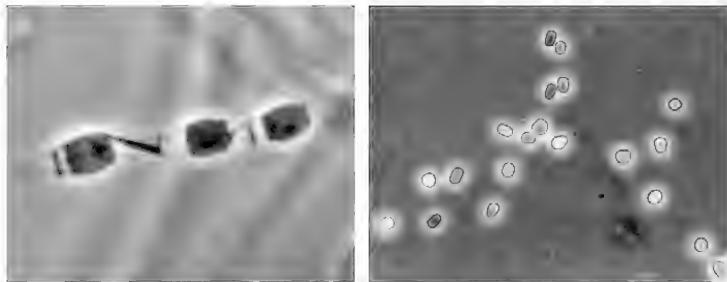
Several authors have reported the occurrence of yellow brown thick walled spores in the flesh of *Gyrodontium* basidiocarp, sometimes forming a dense brown powdery layer (Reid 1963, Maas Geesteranus 1964). Although they interpreted such spores either as chlamydospores or as basidiospores, owing to their close resemblance to true basidiospores, both authors were doubtful about their origin. Study of pure culture allowed to determinate the nature of these spores.

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THE *LACTARIUS GYMNOCARPUS* COMPLEX IN TROPICAL AFRICA

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Abstract. The *Lactarius gymnocarpus* group has been studied as part of a revision of the genus *Lactarius* in tropical Africa.
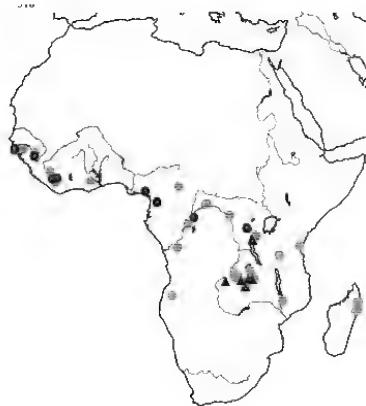
Fig. Collecting sites of *Lactarius gymnocarpus* (●) and *L. longisporus* (▲) and other *Lactarius* species (○).

Detailed study of morphological and anatomical characters combined with ecological data of 99 collections of so called or close to *Lactarius gymnocarpus* Heim maily from African miombo woodland lead us to recognize 6 new species to *Lactarius gymnocarpus*. They are *L. pseudogymnocarpus, L. gymnosporus, L. gymnocarpoides, L. luteopus, L. medusae* and *L. flammans*. The diagnostic differences are illustrated by drawings, photographs and distributions maps.

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SCORIAS SPONGIOSA (SCHW.)FRIES FROM KENYA

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Abstract. This paper describes the "sooty mould" fungus, *Scorias spongiosa* *(*Schw.) Fries from Kenya usually found associated with scale insects of the genus *Coccus.* The fungus is found mainly during dry weather conditions.

AN INTERESTING SPECIES OF *PUCCINIA* ON *GENTIANA*

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Abstract. An interesting specimen of *Puccinia* living on *Gentiana cruciata* L. has been collected near Vikos gorge, at Ipiros, Greece. A detailed study of its morphological characteristics, revealed some remarkable differences from the known species of *Puccinia* on *Gentiana.* It seems to be closely related to *Puccinia gentianae* (F. Strauss) Rohl., which is widespread in the northern hemisphere, but unknown from Greece. A comparative study of our specimen with authentic material of *P. gentianae* is necessary, for the determination of its exact taxonomic position.

It is the first time that the genus *Gentiana* is reported from Greece, as a host of a rust fungus.

CULTURAL CHARACTERISTICS AND THEIR TAXONOMIC POTENTIAL IN *LENTINELLUS* SPECIES

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Abstract. Mycelial cultures of three species of the genus *Lentinellus* Karsten have been studied. The cultural, macroscopical and microscopical, as well as cytological characters of the species and their ability to produce polyphenol oxidases are presented. The aim of this study is not only to give the culture features of the three species but also to get additional viewpoints for a better understanding of the

taxonomic relationships within the genus *Lentinellus*. Thus there is a taxonomic approach in which the study of cultures is intended to contribute to the evaluation of fungal relationships.

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RESPONSES TO THE THERMAL STRESS IN THE INTERSTERILITY GROUPS OF *GANODERMA AUSTRALE* IN TAIWAN

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Abstract. The diversity of responses to thermal stress of two "intersterility groups" of *Ganoderma australe* (Fr.) Pat. in Taiwan was studied and its impact on the migration of tropical fungi to the temperate region in Asia was discussed. The intersterility Group 1 (isolate TAI-01) is a tropical strain distributed in the lowland area of Taiwan and has a higher optimum growth temperature range i.e., 28°-32°C than that of Group 2. The tropical strain responded to 40°C heat shock treatment by synthesizing seven heat shock proteins with mol. wt. of 13.5 -98 kDa. The intersterility Group 2 (isolate TAI-05) is distributed in the high land area of 1.650 m altitude of Taiwan and has an optimum growth temperature range of 24°-28°C but showed no response to heat shock. Instead, it responded to cold shock by synthesizing a cold shock protein of 41 kDa. Evidently the Group 2 of *G. australe* in Taiwan has been reproductively isolated from Group 1 and has adapted to the habitat of the temperate climate and is a temperate strain. The migration of tropical fungi into the temperate zone requires a speciation process and an induction of cold-shock proteins. The subtropical island Taiwan can provide an excellent bridge for the floristic migration between the Torrid and the Temperate species of fungi in Asia.

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MOLECULAR TAXONOMY

18S RRNA AND THE FUNGAL TREE: EVOLUTIONARY RELATIONSHIPS AMONG ASCOMYCETES, BASIDIOMYCETES, ZYGOMYCETES, AND CHYTRIDIOMYCETES.

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Introduction

A valuable and reliable classification of organisms should ideally be based on their natural evolutionary relationships. Although morphology is an inevitable tool for the description of a species, drawing conclusions about its genealogy from morphological or biochemical characteristics is far more difficult. This is especially true for prokaryotes, but also for eukaryotes with simple morphology, like yeasts. But even for organisms with a more pronounced morphology, there can be misinterpretation of the data due to convergent evolution and disagreement about phenotypic characteristics being ancestral or derived.

However, the evolutionary history of an organism is recorded in its genes and the development of sequence analysis techniques has allowed reconstruct biological evolution by comparing the structure of genes or gene-products in different species. One of the molecules exceedingly suited for this purpose is the small ribosomal subunit RNA (further abbreviated as SSU rRNA) or 18S rRNA (in eukaryotes). SSU rRNA, present in all living organisms, as well as in organelles like plastids and mitochondria, has a conserved structure and an average chain length of about 1800 nucleotides in eukaryotes. As a consequence of its popularity in the construction of evolutionary trees, over 3000 complete (or nearly complete) sequences have been determined. They are recorded in our SSU rRNA database, which is made available to the scientific community (Neefs *et al.* 1993). About 700 of these sequences are from eukaryotes and about 165 of these are from "true" fungi, covering a wide range of different genera and families.

Results

Figure 1 shows an evolutionary tree based on all SSU rRNA sequences known to date of ascomycetes, basidiomycetes, zygomycetes, and chytridiomycetes. Only one rRNA sequence is included if several are determined for the same species, usually by different authors. Dissimilarity, being the fraction of substitutions between two sequences, was converted into evolutionary distance by using the equation of Jukes and Cantor (1969), which corrects for multiple mutations per site. The tree topology was inferred by the neighbour-joining method of Saitou and Nei (1987). The complete alignment was used for tree construction. Confidence values for individual branches were determined by a bootstrap analysis in which 100 bootstrap trees were generated from resampled data (Felsenstein 1985). Distance calculation, tree construction and bootstrap analysis were performed with the software package TREECON (Van de Peer and De Wachter 1993).

Distance 0.1

plectomycetes

euascomycetes

loculoascomycetes

pyrenomycetes

discomycetes

hemiascomycetes

uncertain affiliation

ustomycetes

true basidiomycetes

CHYTRIDIOMYCETES

ZYGOMYCETES

ASCOMYCETES

BASIDIOMYCETES

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Phylogeny of Chytridiomycetes and Zygomycetes

In the tree of Fig. 1, the chytridiomycete *Blastocladiella emersonii* and the zygomycete *Mucor racemosus* form the first lines of divergence within the fungi. Next there is a splitting of, on the one hand, Ascomycetes and Basidiomycetes, and on the other hand, the remaining Zygomycetes and Chytridiomycetes. Thus, based on this result, the Chytridiomycetes as well as the Zygomycetes seem polyphyletic. However, since both *Blastocladiella* and *Mucor* form long branches because of a higher evolutionary rate, their divergence should be interpreted with caution. It is a known phenomenon that long branches can be pulled to some extent towards the root of the tree (Olsen 1988), because of an underestimation in the Jukes-Cantor conversion of dissimilarity into evolutionary distance (Golding 1983). Furthermore, until now, only one representative is sequenced for the Blastocladiales and for the Mucorales and it is possible that addition of more representatives of these orders may change and stabilize their respective position in the tree. *Blastocladiella* and *Mucor* not considered, Zygomycetes and Chytridiomycetes are clustered together and seem to form a monophyletic lineage, supported at a fairly high bootstrap level (80%). In this tree, the zygomycete *Endogone pisiformis* is clustered with the Chytridiomycetes, as also noticed by Bruns *et al.* (1992). However, its position is unstable (only supported in 51% of the bootstrap samples) and depends on the composition of the dataset.

Phylogeny of Ascomycetes

Basidiomycetes and Ascomycetes clearly form two separate monophyletic groups based on SSU rRNA data (Bruns *et al.* 1992, Van de Peer *et al.* 1992, Wilmotte *et al.* 1993, this study). Furthermore they are sister groups supported at a high bootstrap level (96%). The Ascomycetes are subdivided into two major lineages: the Euascomycetes and the Hemiascomycetes. Within the Euascomycetes, three clearly separated groups can be distinguished, viz. Plectomycetes, Loculoascomycetes, and Pyrenomycetes, while the Discomycetes are divided over two different lineages. Overall, this subdivision of the Euascomycetes agrees well with classical taxonomic views.

The Hemiascomycete cluster consists of yeasts and yeast-like fungi, most of which were formerly classified as Deuteromycetes. Based on SSU rRNA it is clear that some of these yeast genera like *Candida* and *Pichia*, are heterogenous and it would be meaningful to reconsider their taxonomy. A detailed discussion of many of the species included in both the Euascomycete and the Hemiascomycete cluster is given in Wilmotte *et al.* (1993).

Within the Ascomycetes, a third cluster, although not supported by bootstrap analysis, is formed by the genera *Neolecta*, *Pneumocystis*, *Taphrina*, *Schizosaccharomyces, Protomyces*, and *Saitoella*. The exact taxonomic position of these species is not clear and deserves further attention.

Phylogeny of Basidiomycetes

Within the Basidiomycetes, three main lineages can be discerned, two leading to

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the Ustomycetes and one leading to the true Basidiomycetes, two taxa created by Moore (1972) on the basis of differences in septal ultrastructure and life cycle. According to this tree and some previously published trees based on SSU rRNA (e.g. Wilmotte *et al.* 1993), the Ustomycetes seem to be paraphyletic, although this finding is not supported at a high level by bootstrap analysis.

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A RAPID MOLECULAR TECHNIQUE TO DISTINGUISH
CYLINDROCLADIUM SPECIES

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Introduction

In a recent monograph of *Cylindrocladium* Morgan, Crous and Wingfield (1994) recognized 22 species and 2 varieties. Four of these species, namely *C. clavatum* Hodges & May, *C. pteridis* Wolf, *C. gracile* (Bugn.) Boesewinkel and *C. hawksworthii* Peerally are known to have 1-septate conidia and thin-walled stipe extensions terminating in a clavate vesicle. *C. hawksworthii* can easily be distinguished in having curved conidia, whereas those of *C. clavatum*, *C. gracile* and *C. pteridis* are straight. The latter three species have similar temperature requirements for growth, and are primarily distinguished on cultural characteristics and dimensions of conidia and stipe extensions. These species represent a range in size of conidia and stipe extensions from the smaller *C. clavatum* and *C. gracile* to the larger *C. pteridis* (Crous and Wingfield 1994). *Calonectria* teleomorphs have been described for *C. pteridis*, *C. gracil*e and recently also for *C. clavatum* (Crous *et al.* 1993b, El-Gholl *et al.* 1993). However, an examination of the type strains of *Cylindrocladium clavatum* and *Calonectria clavata* Alfieri *et al.* found them to be morphologically distinct.

Complementing alpha and beta taxonomy, several molecular techniques have recently been employed in *Cylindrocladium*, ranging from total protein and isozyme banding patterns to DNA restriction fragment length polymorphisms (RFLPs) (Crous *et al.* 1993a, 1993c). These techniques proved to be time consuming, and in the case of proteins, often influenced by host and geographical variation. It has been shown, however, that DNA RFLPs can clearly distinguish variation between and among species, and therefore substantially enhance attempts to allocate isolates in *Cylindrocladium* (Crous *et al.* 1993a). In the Hypocreales, ribosomal DNA (rDNA) restriction fragment length polymorphisms (RFLPs) have been successfully used as a rapid technique to demonstrate species-specific differences in *Fusarium* Link (Lodolo *et al.* 1992). The aim of the present study, therefore, was to use this technique to establish variation among the four *Cylindrocladium* species discussed above, and to determine the correct taxonomic position of the type strain of *Calonectria clavata.*

Material and Methods

Morphological characterization

The following type and verified strains of *Cylindrocladium* and *Calonectria* spp. were studied: *Cylindrocladium clavatum* (PPRI 3994 = CPC 328), (ATCC 22833, ex type strain); *Calonectria clavata* (078-1261 = ATCC 66388 and 078-1543 = ATCC 66389, ex type strains); *Cylindrocladium gracile* (PC 551197, ex type strain); *Calonectria gracilis* Crous, Wingfieldand Alfenas (PPRI 4176 = AR 2677, ex type strain), *Cylindrocladium hawksworthii* (MUCL 30866, ex type strain); *Cylindrocladium pteridis* (PPRI 4157 = UFV 43).

Single-conidial isolates were cultured on 2 % malt extract agar (MEA) (Oxoid), plated onto carnation-leaf agar (CLA), incubated at 25 °C under near-ultraviolet light, and examined after 7 d. Only material occurring on carnation leaves was examined. Mounts were prepared in lactophenol cotton blue, and measurements made at 1000 x magnification.

Chromosomal DNA isolation

Single-conidial isolates were grown on MEA, and plugs of seven-day-old cultures transferred into 500 ml Erlenmeyer flasks containing 100 ml glucose-yeast extract broth (Biolab). Cultures were incubated for 7-14 d in the dark at 25 °C until sufficient growth occurred. Mycelia were harvested by filtration (Whatman No. 1 filter paper), the mycelial mat immersed into liquid nitrogen and chromosomal DNA isolated. Chromosomal DNA was subsequently redissolved in 200 µl TE buffer (pH 8.0).

Restriction enzyme analysis and Southern hybridization

Chromosomal DNA (ca. 5 µg) of each isolate was subjected to restriction digestion with EcoRI, HindIII and XhoI for 3 h respectively, according to the recommendation of the suppliers (Boehringer Mannheim). The DNA was separated on horisontal 0.8 % agarose gels and transferred to Hybond-N nylon membranes (Amersham) according to standard procedures. The *Neurospora crassa* rDNA was purified from plasmid pMF2 (Russell *et al.* 1984) as a 6.3 kb PstI fragment and labelled with [‡-32P]dATP (Amersham). The Southern hybridizations and stringency washes were performed according to the method of Sambrook et al. (1989).

Results

Morphological characterization

All the species examined in the present study produced stipe extensions terminating in clavate vesicles. Based on conidium morphology on CLA, two groups could be distinguished. Conidia of *C. clavatum*, *C. gracile* and *C. pteridis* were straight, whereas those of *C. hawksworthii* and Calonectria clavata were curved. Conidia of *Cylindrocladium clavatum* were 38-52 x 4-6 µm, overlapping with the slightly larger conidia of *Cylindrocladium gracile* (PC 551197), which were 40-56 x 3.5-5 µm. Conidia of *Calonectria gracilis* were 40-65 x 4-5 µm, overlapping to some degree with the lower range of *Calonectria pteridis*, which were 62-121 x 5-6 µm. The same trend was also observed for their ascospores, those of *C. gracilis* being 1- septate, (27-)36.5(-50) x (4-)5(-6) µm, and those of *C. pteridis* 1(-3)-septate, (30)-51.5(-75) x (4.5)5.5(-7) µm. Of the two species with curved conidia, those of *C. hawksworthii* (42-76 x 4-4.5 µm) were similar in length, but slightly narrower than those of *Calonectria clavata* (50-80 x 5-6 µm).

Restriction enzyme analysis and Southern hybridization

Using the *Neurospora crassa* rDNA probe five distinct EcoRI, XhoI and HindIII restriction patterns were obtained for the different strains (Figs. 1-3). The South African collection of *Cylindrocladium clavatum* (CPC 328) was similar to that of the type culture of *C. clavatum* (ATCC 22833). However, the type culture of *C. gracile* (PC 551197) could not be distinguished from that of *C. clavatum*. The two heterothallic isolates of *Calonectria clavata* were similar to each other, but distinct from that of *Cylindrocladium clavatum*, and all other species studied. The profile of

Calonectria gracile (AR 2677) was distinct from *Cylindrocladium gracile* (PC 551197), and closer to that of *Cylindrocladium pteridis*.

Discussion

Type and verified strains of four *Cylindrocladium* species were investigated in this study. Three of these have been associated with *Calonectria* teleomorphs, and type strains of the latter were also included. Comparisons were done on the basis of morphology, culture characteristics and nuclear DNA polymorphisms.

Cylindrocladium hawksworthii

The species is known from two collections made in Mauritius (Peerally 1991). It is distinguished from other species in *Cylindrocladium* in having primarily clavate vesicles, and curved 1-septate conidia. The phenomenon of curved macroconidia in *Cylindrocladium* is known from *C. curvatum* Boedijn and Reitsma (1950) with sphaeropedunculate vesicles, and *C. variabile* Crous *et al.* (1993a), with sphaeropedunculate to ellipsoidal or clavate vesicles and (1-)3(-4)-septate conidia. The present study also found the two heterothallic strains of *Calonectria clavata* to have prominently curved macroconidia. The latter strains could, however, be distinguished from *Cylindrocladium hawksworthii* by their larger conidium dimensions and septation, as well as distinct rDNA restriction patterns.

Cylindroladium clavatum and *Cylindrocladium gracile*

C. clavatum was described by Hodges and May (1972) with conidia being 37.5-48 x 3.5-5.5 µm. Bugnicourt (1939) described *Cylindrocarpon gracile* with conidial dimensions as being 24-48 x 2.5-4 µm. Boesewinkel (1982) transferred *Cylindrocarpon gracile* to *Cylindrocladium*, and retained it as a separate species because of its longer stipe extensions and narrowly clavate vesicles.

Results obtained with rDNA restriction patterns for EcoRI, XhoI and HindIII in the present study clearly indicate, however, that the type culture of *Cylindrocladium gracile* (PC 551197) is indistinguishable from that of *C. clavatum* (ATCC 22833). The similarity in morphology and rDNA restriction pattern suggests therefore that the two species are synonymous. Because *gracile* is the older epithet, *C. gracile* Bugnicourt is to be accepted as the earlier name for this species.

Calonectria pteridis (anam. *Cylindrocladium pteridis*) and *Calonectria gracilis*

Of the 1-septate species with clavate vesicles, *C. pteridis* has the largest macroconidia, and frequently also forms curved microconidia in culture. In the present study curved microconidia have also been observed for *Calonectria clavata*. Similar to *C. clavata*, *C. pteridis* has also been found to be heterothallic, with successful matings producing the teleomorph in culture (Crous *et al.* 1993b).

A homothallic strain with conidial dimensions of 40-65 x 4-5 µm was recently obtained from Brazil. Because these dimensions overlapped considerably more with that of *C. gracile* than *C. pteridis*, and *C. pteridis* was known to be heterothallic with larger, 1(-3)-septate ascospores, the isolate was described as the *Calonectria* teleomorph of *Cylindrocladium gracile* (Crous *et al.* 1993b). As stated above, the present study found *Cylindrocladium gracile* to be distinct from *Calonectria gracile*, and synonymous with *Cylindrocladium clavatum*.

The rDNA restriction patterns obtained with EcoRI and HindIII showed only minor differences between *Calonectria gracile* and *Calonectria pteridis*. These two

isolates could, however, easily be distinguished with the restriction enzyme XhoI. Based on the differences in the banding patterns, as well as conidium and ascospore dimensions, it would appear that *Calonectria gracilis* represents a distinct species between *Cylindrocladium clavatum* and *Cylindrocladium pteridis*. We are, however, of the opinion that additional isolates of *Calonectria pteridis* will have to be studied to suitably resolve the validity of *Calonectria gracilis*.
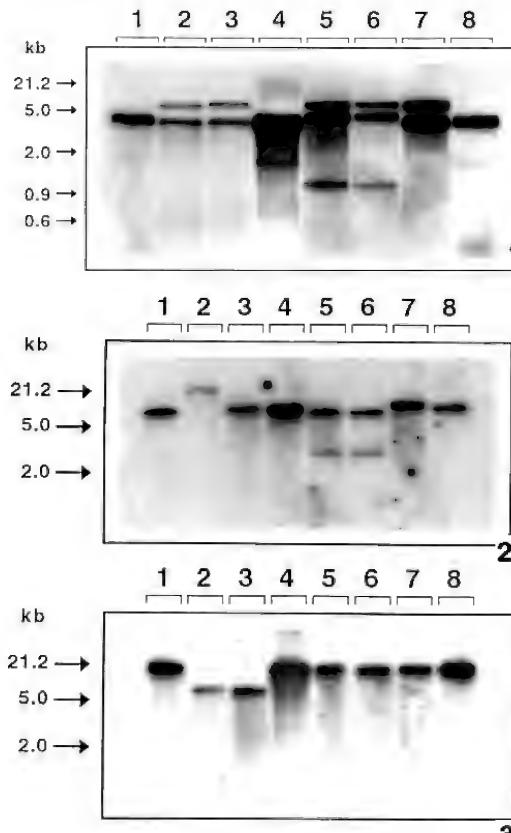
Fig.1. rDNA hybridization patterns for EcoRI-digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Lane 1: *Cylindrocladium clavatum* PPRI 3994. Lane 2: *Calonectria gracilis* PPRI 4176. Lane 3: *C. pteridis* PPRI 4157. Lane 4: *Cylindrocladium gracile* PC 551197. Lanes 5 and 6: *Calonectria clavata* ATCC 66388 and 66289. Lane 7: *Cylindrocladium hawksworthii* MUCL 30866. Lane 8: *C. clavatum* ATCC 22833. Size markers are lambda DNA digested with EcoRI and HindIII.
Fig.2. rDNA hybridization patterns for XhoI-digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Details as in Fig. 1.
Fig.3. rDNA hybridization patterns for HindIII-digested nDNA of strains of *Cylindrocladium* and *Calonectria* species. Details as in Fig. 1

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Calonectria clavata

The two heterothallic strains of *Calonectria clavata* are morphologically distinct from all presently described species of *Cylindrocladium, C. clavatum* included. Furthermore, their rDNA restriction patterns (Figs. 1-3) show these two isolates to be similar, but distinct from all other species investigated. These results therefore suggest that these isolates represent a presently undescribed species of *Cylindrocladium*.

Conclusion

The present study has found rDNA RFLPs to be effective in distinguishing among morphologically similar species of *Cylindrocladium*. Our results further suggest that this is also an excellent technique for validating anamorph teleomorph relationships where the two states have been described from separate collections. With the recently completed monograph of *Cylindrocladium*, results obtained using this technique can now be integrated with alpha and beta taxonomic criteria. This approach would help to determine the morphological and genetic range of species occurring in morphologically similar complexes.

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IDENTIFICATION OF BASIDIOMYCETOUS YEASTS USING WHOLE-CELL PROTEIN ELECTROPHORESIS

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Introduction

There is a continuing need for simple and reliable laboratory procedures to differentiate yeast species. With an increasing number of species it becomes obvious that the conventional diagnostic tests frequently provide insufficient phenotypic characterization to permit unambiguous assignment at the species level. In this study the use of one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis of whole-cell proteins (SDS-PAGE) as a possible method for classification and identification of basidiomycetous yeasts is investigated.

Materiel and methods

Approximately 240 different strains representing at least 70 different species in the basidiomycetous genera *Cystofilobasidium, Cryptococcus, Filobasidium, Filobasidiella, Kondoa, Leucosporidium, Mrakia, Rhodosporidium, Rhodotorula* and *Tremella* were studied. Cells were cultivated in liquid cultures and harvested in the stable growth phase. The methods used for preparation and electrophoresis of the SDS-protein extracts were those described previously (Vancanneyt *et al.* 1992b). Protein patterns were scanned and digitized. Numerical analysis was performed using the Pearson product moment correlation coefficient and UPGMA clustering.

Results

Except for the genus *Mrakia*, all species of the teleomorphic genera *Cystofilobasidium, Filobasidium, Filobasidiella, Kondoa, Leucosporidium, Mrakia* and *Rhodosporidium* constituted separate protein electrophoretic clusters (Vancanneyt *et al.* 1992a). The species of the genus *Mrakia (M. frigida, M. gelida, M. nivalis* and *M. stokesii)* showed highly similar protein patterns, suggesting that these four species may be synonymous. Strains of two varieties of *Filobasidiella neoformans, F. neoformans* var. *neoformans* and *F. neoformans* var. *bacillispora* could not be differentiated, neither by protein electrophoresis, visually, or by numerical analysis.

A study of 107 *Rhodosporidium* and *Rhodotorula* strains revealed after numerical analysis of protein electrophoregrams eighteen clusters (Vancanneyt *et al.* 1992b). Only the type strain was included for the following ten species: *Rhodotorula armeniaca, Rt. auriculariae, Rt. bacarum, Rt. bogoriensis, Rt. diffluens, Rt. hordea, Rt. hylophila, Rt. ingeniosa, Rt. muscorum* and *Rt. philyla.* All these type strains demonstrated a qualitatively well-characterized protein profile, which might indicate that each of these species occupies a unique and separate taxonomic position within the genus *Rhodotorula.* Homogeneous and separate protein clusters were found for strains of the species *Rt. fragaria, Rt. javanica, Rt. lactosa, Rt. pustula* and *Rt. sonckii.* Intraspecific heterogeneity was demonstrated

within the following seven *Rhodotorula* species: *Rt. acheniorum, Rt. araucariae, Rt. aurantiaca, Rt. foliorum, Rt. glutinis, Rt. graminis* and *Rt. minuta*. One or more strains of the anamorphic species *Rt. glutinis* grouped with strains of *Rs. sphaerocarpum, Rs. toruloides, Rs. diobovatum* strains, *Rs. kratochvilovae* and *Rt. mucilaginosa* strains. For *Rt. graminis*, the type strain grouped with *Rs. diobovatum* strains and most of the strains investigated grouped with *Rs. paludigenum* strains.

Table 1: DNA base composition and type of coenzyme Q within protein electrophoretic groups of *Rhodotorula* and *Rhodosporidium* strains.

Protein group	Species	Number of strains	mol %(G+C)	Coenzyme Q
1	*Rt. glutinis*	2	59.6-60.7	Q10
	Rt. mucilaginosa	15		
2	*Rt. glutinis*	1	62.0-63.0	Q10
	Rs. sphaerocarpum	4		
3	*Rt. glutinis*	1	59.9-60.0	Q9
	Rt. sp.	1		
	Rs. toruloides	6		
4	*Rt javanica*	2	ND	Q9
5	*Rt. graminis*	4	62.5-63.0	Q10
	Rs. paludigenum	4		
6	*Rt. pustula*	2	ND	ND
7	*Rt. aurantiaca*	1	61.3	Q10
	Rt. graminis	1		
8	*Rt. glutinis*	7	66.4-67.2	Q10
	Rt. graminis	1		
	Rs. diobovatum	5		
9	*Rt. glutinis*	2	64.2-65.0	Q10
	Rs. kratochvilovae	1		
10	*Rt. fragariae*	2	ND	Q10
11	*Rt. acheniorum*	2	52.8	Q10
12	*Rs. dacryoidum*	3	ND	ND
13	*Rt. aurantiaca*	2	58.4-59.3	Q10
14	*Rt. aurantiaca*	2	55.1	Q10
15	*Rt. minuta*	10	48.6-51.0	Q10
16	*Rt. lactosa*	2	56.6	Q9
17	*Rt. sonckii*	2	ND	Q10
18	*Rt. aurantiaca*	2	57.6-57.9	Q9

ND: not determined

A study of 78 *Cryptococcus* strains, 3 *Filobasidium* strains and 10 *Tremella* reference strains revealed after numerical comparison eighteen protein electrophoretic clusters (Vancanneyt *et al.* 1994). Only the type strain was included for the following *Cryptococcus* species: *Cr. curiosus, Cr. dimennae, Cr. flavus, Cr. heveanensis, Cr. magnus, Cr. marinus, Cr. skinneri* and *Cr. tsukubaensis*. These type strains showed a well-characterized protein fingerprint. Homogeneous and separate protein clusters were found for strains of the species *Cr. amylolentus, Cr. curvatus, Cr. gastricus* and *Cr. macerans*. A significant protein electrophoretic heterogeneity was observed within the species *Cr. albidus, Cr. humicola, Cr. laurentii* and *Cr. luteolus*. Highly similar protein patterns were observed for some strains of *Cryptococcus albidus* and the investigated strains of *Cr. kuetzingii*, for the

type strain of *Cr. elinovii* and the studied strains of *Cr. terreus* and for a strain of *Cr. luteolus* and two strains of *Cr. laurentii*. Concerning the reference strains of the genus *Tremella, T. aurantia, T. brasiliensis* and *T. globospora* showed a unique protein pattern. A highly similar protein fingerprint was found between one strain of *Cr. Laurentii* and *T. foliacea*. A qualitatively and quantitatively highly similar protein pattern was found for respectively *T64. coalescens* and *T. mesenterica, T. fuciformis* and *T. samoensis* and *T. encephala* and *T. subanomala.*

The taxonomic validity of the obtained groupings was evaluated using other chemotaxonomical criteria such as the determination of the DNA base composition and the ubiquinone type (Table 1 and 2; Vancanneyt *et al.* 1992a, b, 1994). These data confirmed the protein electrophoretic heterogeneity within several anamorphic *Rhodotorula* and *Cryptococcus* species and indicated a possible close relationship between strains of different species.

Table 2: DNA base composition and type of coenzyme Q within protein electrophoretic groups of *Cryptococcus, Filobasidium* and *Tremella* strains.

Protein group	Species	Number of strains	mol %(G+C)	Coenzyme Q
1	*Cr. curvatus*	6	56.7-56.9	Q10
2	*Cr. amylolentus*	2	ND	Q9
3	*Cr. laurentii*	2	54.7-55.2	Q10
	Cr. luteolus	1		
4	*Cr. laurentii*	3	57.2-58.1	ND
5	*Cr. elinovii*	1	54.7-55.0	Q10
	Cr. terreus	3		
6	*Cr. albidus var. aerius*	1	53.7-54.2	Q10
	Cr. albidus var. albidus	1		
7	*Cr. albidus var aerius*	2	49.8-50.1	ND
8	*Cr. albidus var. albidus*	7	51.3-53.0	Q10
	Cr. kuetzingii	4		
9	*Cr. albidus var. albidus*	4	49.1-49.6	Q10
10	*T. encephala*	1	47.6-47.7	Q10
	T. subanomala	1		
11	*T. fuciformis*	1	55.9-56.1	Q10
	T. samoensis	1		
12	*Cr. albidus var. albidus*	3	52.8-53.5	Q10
13	*Cr. ater*	2	51.3-52.0	Q10
	F. foliforme	3		
14	*Cr. humicolus*	2	57.4	ND
15	*Cr. gastricus*	2	ND	ND
16	*Cr. macerans*	4	ND	ND
17	*T. coalescens*	1	46.4-47.2	ND
	T. mesenterica	1		
18	*Cr. humicolus*	8	61.1-62.3	ND

ND: not determined

Conclusion

We conclude that SDS-PAGE of cellular proteins allows a rapid and reliable grouping of a large number of yeast strains. The majority of the species are delineated as separate clusters. The technique is successful in delineating anamorph/teleomorph relations, in revealing synonymy between species and in demonstrating taxonomic heterogeneity within several species. Moreover, the possibility of computerized processing of the patterns suggests a method useful for analytical characterization of yeasts. Identification of new isolates becomes an easy routine procedure once a database of reference protein electrophoregrams is constructed.

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PHYTOPATHOGENIC FILAMENTOUS (*ASHBYA, EREMOTHECIUM*) AND DIMORPHIC FUNGI (*NEMATOSPORA*) WITH FALCATE ASCOSPORES AS NEW MEMBERS WITHIN THE SACCHAROMYCETACEAE.

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Introduction

Although geneticists and molecular biologists commonly separate yeasts and filamentous fungi, Meyen, 1838 has already acknowledged in the name *Saccharomyces* the fact that yeasts are fungi (van der Walt 1987). Wickerham (1951, 1952) concluded that the classification of yeasts will be incorrect as long as the related filamentous forms had been studied thoroughly by persons who knew these fungi as well as yeasts.

In the present work we investigated the phylogenetic relationship between the saprophytic and predominantly unicellular yeast genera *Saccharomyces* and *Kluyveromyces* and the plant pathogenic, filamentous fungi *Ashbya gossypii* and *Eremothecium ashbyi*. Dimorphic yeast, *Nematospora coryli*, parasitic on hazelnuts and soybeans was included in our investigations. To characterize yeasts and filamentous fungi from these genera on the molecular level we have chosen the cell wall monosaccharide composition, the presence of DL-dityrosine-containing macromolecules from ascospore walls (Briza *et al.* 1990), the ubiquinone spectra, and the DNA sequence information from the small and large ribosomal subunit genes as well as from the rapidly evolving ITS1 and ITS2 regions.

Material and Methods

Cell wall sugars/Ubiquinone-system. The qualitative and quantitative monosaccharide pattern of purified yeast and hyphal cell walls and ubiquinone spectra were determined as described in Prillinger *et al.* (1993) and Messner *et al.* (1994a). Dityrosine analysis was performed according to Briza *et al.* (1994). Analysis of ribosomal DNA-sequences: Sequences were obtained by direct, automatic sequencing of PCR products (Codon Genetic Systems, Vienna; Messner *et al.* 1994b).

Results and Discussion

Ascospore morphology and the presence of hyphal growth were considered to separate the genera *Ashbya*, *Eremothecium*, and *Nematospora* at least on the family level (Metschnikowiaceae: von Arx and van der Walt 1987) from other yeast genera belonging to the Endomycetales. The ascospores of Saccharomyces species are smooth and spherical, in *Kluyveromyces* they are reniform. Falcate ascospores, often whiplike at one end and occasionally septate are known in *Nematospora coryli*, *Ashbya gossypii* and *Eremothecium ashbyi* (von Arx and van der Walt 1987).

Kurtzman (1993), Kurtzman and Robnett (1994), and Prillinger *et al.* (1990, 1993) presented molecular evidence that the Endomycetales except the Schizosaccharomycetaceae are homogenous. The Schizosaccharomycetaceae were

included in the new order Schizosaccharomycetales. According to Kurtzman & Robnett (1994) and Prillinger *et al.* (1993) representatives of the Endomycetales like *Ashbya*, *Eremothecium*, *Kluyveromyces*, *Nematospora*, and *Saccharomyces* investigated in this study are primitive fungi ancestral to filamentous Asco- and Basidiomycetes.

To clarify the phylogenetic relationship between the saprophytic unicellular species of *Kluyveromyces* and *Saccharomyces* on the one hand and the phytopathogenic dimorphic (*Nematospora*) or filamentous *Ashbya* or *Eremothecium* species on the other hand we used a polyphasic molecular approach. In the present paper we have investigated:

1. The qualitative and quantitative **monosaccharide composition** of purified yeast and fungal cell walls after hydrolysis with trifluoroacetic acid.
2. The presence of **dityrosine** in the cell walls of ascospores.
3. The major component of the ubiquinone system.
4. The **DNA sequence** of six regions of the **ribosomal RNA genes**.

The cell wall carbohydrate pattern of the phytopathogenic filamentous fungi *A. gossypii* and *E. ashbyi* comes close to the characteristic mannose-glucose pattern of several *Saccharomyces* and *Kluyveromyces* species (Prillinger *et al.* 1990). Whereas the proportion of mannose appeared to be commonly higher in the *Kluyveromyces* and *Saccharomyces* yeast strains as well as in *N. coryli*, glucose dominates in the filamentous strains of *A. gossypii* and *E. ashbyi*.

As far as ascospores could be observed on different sporulation media (Kreger-van Rij 1984), dityrosine was detected in all species of *Saccharomyces*, *Kluyveromyces*, and the two filamentous fungi *A. gossypii* and *E. ashbyi*. Dityrosine was absent in sporulating cultures from *Nadsonia fulvescens* (Ubiquinone Q-6), *Debaryomyces hansenii* (Q-9), *Pichia farinosa* (Q-9), and *Schizosaccharomyces pombe* (Q-10).

As already shown by Yamada *et al.* (1977, 1987), the ubiquinone Q-6 was present in all species of the genera *Kluyveromyces* and *Saccharomyces* as well as in *A. gossypii* and *E. ashbyi*. Ubiquinone Q-5 was found in the type strain of *Nematospora coryli*. According to Yaada *et al.* (1987) there is an additional strain of *N. coryli* where ubiquinone Q-6 was found. Conspecificity was established for both strains based on partial base sequences of 18S and 26S ribosomal DNA (Yamada and Nagahama 1991).

For molecular phylogeny based on DNA sequences three different targets were chosen. Regions of 800 to 900 bases length of the genes for the small and large ribosomal subunit as well as both the complete internal transcribed spacers ITS1 and ITS2 had been sequenced (Messner *et al.* 1994b).

Separate phylogenetical computation of these three types of ribosomal sequences results in the clustering of the *Saccharomyces* species, the *Kluyveromyces* species, *E. ashbyi*, *N. coryli*, and *A. gossypii* for all regions investigated. As illustrated by Fig. 1 deriving from ITS1 and ITS2 data, the genera *Saccharomyces* and *Kluyveromyces* are well defined by 82 and 98 % bootstrap confidence, respectively. *E. ashbyi*, *N. coryli* and *A. gossypii* are clustered here at the species level, and the separation of these three subclades from *Candida* by tree topology and a bootstrap value of 65 % is quite good for sequences of the rapidly evolving ITS regions.
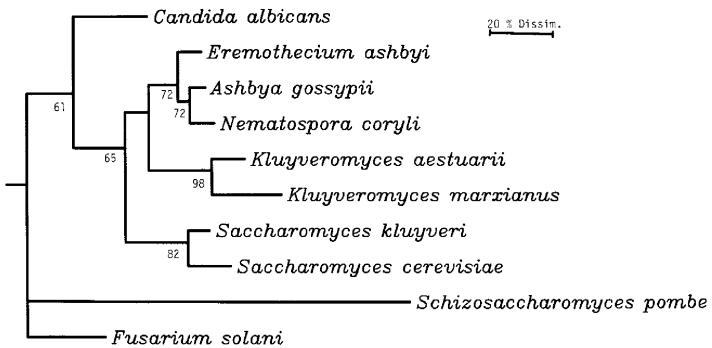
Fig. 1. Cladogram based on both internal transcribed spacer regions ITS1 and ITS2. An alignment of 655 bases in length was computed under maximum likelihood hypothesis. Bootstrap confidence values derived from 600 repeats.

Our data corroborate the work of Kurtzman and Robnett (1994), who found by ribosomal sequences a close relationship between *Ashbya*, *Eremothecium*, and *Nematospora* together with *Holleya* tight enough to be congeneric. The genus *Metschnikowia*, however, appears to be only distantly related according Kurzmann and Robnett (1994).

Conclusions

1. Phenotypic criteria like ascospore shape and ornamentation or presence or absence of hyphae are in most cases unreliable for definition of families in the Endomycetales.

2. Based on a similar cell wall carbohydrate composition, the presence of dityrosine in endospores, which are formed by free cell formation, and a high degree of ribosomal DNA sequence similarity especially with respect to the ITS1 and ITS2 regions we redefine the family Saccharomycetaceae Winter to include unicellular saprophytic fungi like the genera *Kluyveromyces* and *Saccharomyces*, as well as dimorphic or filamentous parasitic fungi like species of the genera *Ashbya*, *Eremothecium*, *Holleya*, and *Nematospora*.

3. Our data suggest that unicellular ascomycetous yeasts like *Kluyveromyces* and *Saccharomyces* have evolved from filamentous plant pathogens with ontogenetic saprophytic yeast stages. The lack of the respective parasitic filamentous forms can be explained by an extinction of the specific hosts.

4. Molecular characteristics like cell wall sugars or ribosomal DNA sequence information are reliable tools to trace ascomycetous yeasts back to a polykaryotic coenocytic ("siphonal") ancestor (Prillinger 1987).

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GENETIC ORGANIZATION OF THE YEAST *YARROWIA LIPOLYTICA*

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Introduction

Yarrowia lipolytica (otherwise known as *Candida lipolytica* and *Saccharomycopsis lipolytica*) is a dimorphic yeast which forms both yeast-like cells and true mycelium. It can grow on hydocarbons and has been used for the production of single-cell protein and citric acid as well as various other various metabolites. In addition, *Y. lipolytica* is able to secrete naturally a number of enzymes in large amount. These characteristics make this yeast an organism with high biotechnogical potential.

Although sexuality in this yeast was discovered more than 20 years ago, genetic studies were hampered by unusual features including low mating frequencies, low fertility of hybrids, irregular meiotic segregation and mitotic haploidization. Despite the development of programs to generate numerous mutants and to improve mating procedures and tetrad analysis (Gaillardin *et al*. 1973, Ogrydziak *et al*. 1978), the construction of a genetic map did not allow the detection of any centromere-linked genes and the number of chromosomes in this yeast.

Recent studies in our laboratory (Naumova *et al*. 1993) have defined, using Pulse Field Gel Electrophoresis (PFGE), the existence of three groups of electrophoretic karyotypes amongst 27 natural isolates. Moreover, within these groups an important variability of the number of chromosomes (from 3 to 6 varying in size from 2 to 6 Mb) was observed as well as an important chromosomal-length polymorphism. Hybridization studies with few cloned genes to these karyotypes revealed that these markers were not linked to specific chromosomes when strains were compared. This result is in agreement with the absence of centromere linkage observed with tetrad analysis during the establishment of the genetic map (Ogrydziak *et al*. 1982). Taken together, these results could account for the genetic anomalies displayed by this species.

In a different approach based on the separation of chromosomes by PFGE and the assignement of cloned genes to these chromosomes, we set up the construction of a physical map of the genome of *Y. lipolytica*, in order to facilitate genetic studies and to gain better insights on the unusual genetic characteristics of this yeast.

Material and Methods

Strains used in this study

E150	lab. strain, from the crossing W29 x CBS6124-2	CLIB 122
E129	lab. strain, from the crossing W29 x CBS 6124-2	CLIB 121
H222	lab. strain from H. Weber (Germany)	CLIB 80
CX161-1B	lab. strain from D.M. Ogrydziak (U.S.A.)	CLIB 153
W29	wild type, ATCC 20460	CLIB 89
CBS 6124-1	monosporous segregant from the wild type CBS 6124	CLIB 77
CBS 6124-2	monosporous segregant from the wild type CBS 6124	CLIB 78
B204-12D	lab. strain from G. Barth (Swizerland)	

Preparation of chromosomal DNA:
     This was essentially performed as described by Naumova *et al.* (1993).

Chromosome separation
     Chromosomes were separated in 0.8% agarose gels run in 0.5xTAE buffer at 12°C in a Bio-Rad CHEF-DRII. Electrophoresis was carried out at 50V for 48hrs with a switching time of 2400s, 43V for 70hrs with a switching time of 3000s and 40V for 47.7hrs with a switching time of 3300s.

Southern blotting and DNA/DNA hybridization
     DNA from agarose was depurinated, denaturated and alkali-transferred overnight to GeneSceen membranes. The membranes were then neutralized.
DNA used as probe was directly ^{32}P-dCTP labelled with the random priming method (MegaPrime, Amersham) in low-melting agarose or after purification from agarose gel with the GeneClean kit. Hybridizations were performed at 65 °C in 3xSSPE with Denhardt and dextransulfate. The final wash was in 40mM Na-phosphate/1% SDS at 65 °C.

Results and Discussion
     Important efforts were devoted to the improvement of *Y. lipolytica* chromosomes separation by PFGE and to the transfer of DNA to membranes in Southern blotting. For this purpose, a laboratory strain, E150, was chosen and we were able to show that this strain possesses at least 5 chromosomes whose sizes vary from 2.6 to 4.9 Mb using the chromosomes of *Schizosaccharomyces pombe* as size markers. The size of the *Y. lipolytica* genome is thus estimated at 18.1 Mb.
     36 genes, mostly cloned in the Laboratoire de Génétique Moléculaire et Cellulaire, others kindly provided by foreign laboratories, were used as probes in hybridization experiments to the PFGE separations. This allowed the obtention of 5 linkage groups comprising:
          - 2 markers on Chrom. I
          - 6 markers on Chrom. II
          - 12 markers on Chrom. III
          - 7 markers on Chrom. IV
          - 11 markers on Chrom. V.
     It must be pointed at that:
(1) 3 centromeres were used as probes defining the chromosomes I, III and IV. This allows for the first time, a certain number of markers to be linked to centromeres.
(2) rDNA clusters were shown, unlike other yeasts, to be present on 4 chromosomes out of 5. It is remarkable that, unlike other yeasts too, *Y. lipolytica* possesses at least 2 classes of rDNA units (Fournier *et al.* 1986). The rDNA clusters being highly variable in size, their presence on most of the chromosomes of the strain tested could explain the chromosome-length polymorphism observed in different *Y. lipolytica* strains.
     In order to validate the construction of a physical map of the genome, we tested the distribution of the already assigned markers in the E150 strain to a number of widely used wild type and laboratory strains.
     First, electrophoretic karyotypes of 7 different isolates were obtained. This allowed us to confirm the results obtained by Naumova *et al.* (1993) on the

important variability of the genome structure of realy related *Y. lipolytica* isolates. Then, a pool of genes (*Bio6*, *His1*, *Pho2*, *PGK*) known to hybridize to the E150 chromosome III was used as probes to hybridize to the chromosome separations of the 7 isolates. Preliminary results indicate that the linkage previously obtained with the 4 genes in E150 was not conserved in these strains, including in the parental strains of E150. Indeed, whereas only one signal was observed on the chromosome III of E150, hybridization signals were scattered over the separations of the other strains.

Surprisingly, in the same test performed with a marker of the E150 chromosome I, the *Ryl2* gene, a single signal on the smallest chromosome of each separation was observed, indicating that this chromosome did not display important length polymorphism or gross rearrangement from strain to strain. This is to be linked to the fact that the chromosome I is not carrying rDNA clusters unlike the other chromosomes.

These observations lead us to incriminate homologous recombination between the rDNA clusters on most of the chromosomes to explain the chromosomal rearrangements and the specific genetic features of *Y. lipolytica*. This hypothesis is being tested through the study of the conservation of linkage groups at meiosis and a better characterization of the location of the rDNA clusters in respect with the assigned markers.

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TAXONOMIC REVISION OF *SACCHAROMYCES* SENSU STRICTO STRAINS OF NCAIM

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Introduction

In the last decades, classification of species in the *Saccharomyces sensu stricto* group have been a hot issue (Lodder 1970, Kreger-van Rij 1984, Barnett 1992). Using conventional identification tests for the determination of phenotypic characters delimitation of species within the group cannot be done satisfactorily. On the basis of nDNA/nDNA reassociation studies, recently four species have been reestablished, namely *Saccharromyces cerevisiae, S. paradoxus, S. bayanus*, and *S. pastorianus* (Vaughan-Martini and Martini 1987, 1989, 1993). In 1990, Rodrigues de Sousa *et al.* investigated the fructose proton symport activity in species of *Saccharomyces sensu stricto* group. They reported that four strains of *S. pastorianus* and five strains of *S. bayanus* tested showed fructose proton symport activity whereas five strains of *S. cerevisiae* and three strains of *S. paradoxus* did not. They suggested that active fructose transport is a reliable phenotypic character that correlates with species delimitation based on the genotype.

The aim of our study was to check the reliability of the active fructose proton symport in differentiating 72 species of *Saccharomyces sensu stricto*, using yeast strains isolated from Hungarian wine and beer.

Material and Methods

Yeast strains

The type strains of Saccharomyces sensu stricto species and some of their synonyms and 72 wine and brewer yeast strains identified earlier with species belonging to *Saccharomyces sensu stricto* were studied. Thirteen type strains were obtained from the Industrial Yeast Collection of the Dipartimento di Biologia Vegetale, Universita di Perugia, Italy (DBVPG) and the Culture Collection Unit, Fermentation Section, Northern Utilization Research Branch, U.S. Department of Agriculture, Peoria, Illinois, USA (NRRL). The other strains were obtained from the National Collection of Agricultural and Industrial Microorganism, Budapest, Hungary (NCAIM).

Yeast strains were characterized and identified according to methods and keys of Kreger-van Rij (1984) and Barnett *et al.* (1990).

Measurement of fructose transport

Detection of active fructose transport system was performed according to the method of Rodrigues de Sousa *et al.* (1990). Yeast strains were propagated in a liquid mineral medium (van Uden 1967) with 0.5% (w/v) fructose (Merck). Proton symport activity was tested by recording the alkalinization of an aqueous cell suspension after addition of fructose to a final concentration of 10 mmol l-1 using a RADELKIS OP-211/1, (Hungary) pH meter (precision ±0.05 pH) with OP-0808P pH sensitive combination glass electrode, and registered with a Potentiometric OH-814/1 type recorder (RADELKIS, Hungary).

Table 1. Active fructose transport of yeast strains belonging to *Saccharomyces* sensu stricto group

Species original epithet	Collection numbers	Isolation source	Fructose H+ symport
Saccharomyces cerevisiae			
Sacch. cerevisiaeT	DBVPG 6173	beer	-
Sacch. cerevisiae	NCAIM Y.00201	wine	-
Sacch. cerevisiae	NCAIM Y.00204	wine	-
Sacch. cerevisiae	NCAIM Y.00205	wine	-
Sacch. cerevisiae	NCAIM Y.00206	wine	-
Sacch. cerevisiae	NCAIM Y.00222	wine	-
Sacch. cerevisiae	NCAIM Y.00223	wine	-
Sacch. cerevisiae	NCAIM Y.00225	wine	-
Sacch. cerevisiae	NCAIM Y.00227	wine	-
Sacch. cerevisiae	NCAIM Y.00228	wine	-
Sacch. cerevisiae	NCAIM Y.00235	wine	-
Sacch. cerevisiae	NCAIM Y.00237	wine	-
Sacch. cerevisiae	NCAIM Y.00238	wine	-
Sacch. cerevisiae	NCAIM Y.00248	wine	-
Sacch. cerevisiae	NCAIM Y.00249	wine	-
Sacch. cerevisiae	NCAIM Y.00252	wine	-
Sacch. cerevisiae	NCAIM Y.00254	wine	-
Sacch. cerevisiae	NCAIM Y.00255	wine	-
Sacch. cerevisiae	NCAIM Y.00256	wine	-
Sacch. cerevisiae	NCAIM Y.00259	wine	-
Sacch. cerevisiae	NCAIM Y.00261	wine	-
Sacch. cerevisiae	NCAIM Y.00294	wine	-
Sacch. cerevisiae	NCAIM Y.00295	wine	-
Sacch. cerevisiae	NCAIM Y.00297	wine	-
Sacch. cerevisiae	NCAIM Y.00299	wine	-
Sacch. cerevisiae	NCAIM Y.00301	wine	-
Sacch. cerevisiae	NCAIM Y.00303	wine	-
Sacch. cerevisiae	NCAIM Y.00304	wine	-
Sacch. cerevisiae	NCAIM Y.00305	wine	-
Sacch. cerevisiae	NCAIM Y.00307	wine	-
Sacch. cerevisiae	NCAIM Y.00309	wine	-
Sacch. cerevisiae	NCAIM Y.00312	wine	-
Sacch. cerevisiae	NCAIM Y.00368	wine	-
Sacch. cerevisiae	NCAIM Y.00370	wine	-
Sacch. cerevisiae	NCAIM Y.00378	wine	-
Sacch. cerevisiae	NCAIM Y.00417	wine	-
Sacch. cerevisiae	NCAIM Y.00418	wine	-
Sacch. cerevisiae	NCAIM Y.00419	wine	-
Sacch. cerevisiae	NCAIM Y.00420	wine	-
Sacch. cerevisiae	NCAIM Y.00424	wine	-
Sacch. cerevisiae	NCAIM Y.00425	wine	-
Sacch. cerevisiae	NCAIM Y.00426	wine	-
Sacch. cerevisiae	NCAIM Y.00427	wine	-
Sacch. cerevisiae	NCAIM Y.00429	wine	-
Sacch. cerevisiae	NCAIM Y.00431	wine	-
Sacch. cerevisiae	NCAIM Y.00442	wine	-
Sacch. cerevisiae	NCAIM Y.00445	wine	-
Sacch. cerevisiae	NCAIM Y.00768	wine	+*
Sacch. cerevisiae	NCAIM Y.00769	wine	+*
Sacch. cheresiensis	NCAIM Y.00817	wine	-
Sacch. prostoserdovii	NCAIM Y.00818	wine	-
Sacch. beticus	NCAIM Y.00819	wine	-

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Saccharomyces bayanus

Sacch. bayanusT	DBVPG 6171	beer	+
Sacch. globosusT	NRRL-Y 12645	pear juice	+
Sacch. heterogenicusT	NRRL-Y 1354	apple juice	+
Sacch. inusitatusT	NRRL-Y 12648	beer	+
Sacch. abuliensisT	NRRL-Y 11845	Mesophylax adopersus	+
Sacch. bayanus	NCAIM Y.00500	wine	-*
Sacch. bayanus	NCAIM Y.00826	wine	-*
Sacch. bayanus	NCAIM Y.00827	wine	-*
Sacch. bayanus	NCAIM Y.00828	wine	-*
Sacch. bayanus	NCAIM Y.00829	wine	-*
Sacch. bayanus	NCAIM Y.00830	wine	-*
Sacch. heterogenicus	NCAIM Y.00831	wine	-*

Saccharomyces pastorianus

Sacch. pastorianus	NT DBVPG 6047	beer	+
Sacch. carlsbergensis	NCAIM Y.00820	beer	+
Sacch. carlsbergensis	NCAIM Y.00821	beer	+
Sacch. carlsbergensis	NCAIM Y.00822	beer	+
Sacch. carlsbergensis	NCAIM Y.00832	beer	+
Sacch. carlsbergensis	NCAIM Y.00833	beer	+
Sacch. carlsbergensis	NCAIM Y.00834	beer	+
Sacch. carlsbergensis	NCAIM Y.00770	beer	-*
Sacch. carlsbergensis	NCAIM Y.00835	beer	-*
Sacch. uvarum	NCAIM Y.00823	beer	-*
Sacch. pastorianus	NCAIM Y.00824	beer	-*

Saccharomyces paradoxus

Sacch. paradoxusNT	DBVPG 6411		-
Sacch. paradoxus	NCAIM Y.00223		-
Sacch. paradoxus	NCAIM Y.00399		-
Sacch. paradoxus	NCAIM Y.00825		-

T = Type strain; NT = Neotype strain; *= Differing results. Strains have been examined for nDNA/nDNA homology, see Table 2; NCAIM = National Collection of Agricultural and Industrial Microorganisms; DBVPG = Industrial yeast collection of the Dipartimento di Biologia Vegetale, Università di Perugia, Italy; NRRL = ARS Culture Collection, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illionis, USA

Table 2. Results of nDNA/nDNA homology %

	Sacch. cerevisiaeT DBVPG 6173	Sacch. pastorianusT DBVPG 6047	Sacch. bayanusT DBVPG 6171
Sacch.cerevisiae NCAIM Y.00768	52	98	-
Sacch.cerevisiae NCAIM Y.00769	50	97	-
Sacch.carlsbergensis NCAIM Y.00770	96	50	-
Sacch.carlsbergensis NCAIM Y.00835	98	48	-
Sacch. uvarum NCAIM Y.00823	89	45	-
Sacch. pastorianus NCAIM Y.00824	94	42	-
Sacch. bayanus NCAIM Y.00826	96	-	18
Sacch. bayanus NCAIM Y.00827	94	-	19
Sacch. bayanus NCAIM Y.00828	90	-	17
Sacch. bayanus NCAIM Y.00829	88	-	22
Sacch. bayanus NCAIM Y.00830	89	-	20
Sacch. heterogenicus NCAIM Y.00831	90	-	12

T = Type strain; NCAIM = National Collection of Agricultural and Industrial Microorganisms
DBVPG = Industrial yeast collection, Dipartimento di Biologia Vegetale, Universita di Perugia,Italy

nDNA/nDNA reassociation studies

Extraction and purification of nDNA was accomplished by a combination of procedures by Marmur (1961) and Price *et al.* (1978). nDNA/nDNA reassociation was determined spectrophotometrically using a Gilford Response 2 instrument with a thermoprogrammer, according to Kurtzman *et al.* (1980). nDNA/nDNA homology % was calculated from the Cot curves (Britten and Kohne 1968) using the formula of Seidler and Mandel (1971).

Results and Discussion

Presence or absence of active fructose transport in 72 yeast strains, which were identified earlier as belonging to the *Saccharomyces sensu stricto* group and in eight *Saccharomyces* type strains are summarized in Table 1. Results obtained with type strains corresponded in all cases with those of Rodrigues de Sousa *et al.* (1990). Thirteen out of 72 wine and brewer strains differed in active fructose transport ability from that expected for the species identified earlier. These strains were examined for deoxyribonucleic acid relatedness. Results of nDNA/nDNA reassociation studies are summarized in Table 2.

Results of active fructose transport investigation in accordance with results of nDNA/nDNA homology confirmed that species of *Saccharomyces sensu stricto* can be divided into two groups: active fructose transport is present in *S. bayanus* and *S. pastorianus* and active fructose transport is absent in *S. cerevisiae* and *S. paradoxus* in agreement with finding of Rodrigues de Sousa *et al.* (1990). Vaughan-Martini and Martini (1993) by testing 12 strains of the four species also confirmed that they can be discriminated on the basis of this property. Molecular taxonomy has reformed yeast classification and has become of prime importance in the differentiation of species (Kurtzman 1987). While nDNA/nDNA reassociation method is an excellent research tool, it is too expensive and sophisticated for routine yeast identification. Active fructose transport can be determined easily and this phenotypic character correlates with the molecular classification of species in the group of *Saccharomyces sensu stricto*. Hence it is a useful method for the identification of yeast strains belonging to *Saccharomyces sensu stricto*. Investigations are in progress to find appropriate and reliable conventional physiological tests as suggested by Vaughan-Martini and Martini (1993) to differentiate between pairs of *Saccharomyces* species differentiated on the basis of the presence or absence of active fructose transport.

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PHYLOGENIC RELATIONSHIPS WITHIN THE *SACCHAROMYCES CEREVISIAE* COMPLEX SPECIES.

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Introduction

Saccharomyces cerevisiae, as conceived by Kreger-van Rij (1984), is a large species covering 75 differently named taxa (*S. cerevisiae* and 75 synonyms). That is the taxon she called *Saccharomyces cerevisiae sensu lato.*

Vaughan-Martini and Martini (1987) demonstrated by DNA/DNA reassosiation the weak relatedness between the type or neotype strains of three of the synonymyzed taxa (*S. pastorianus, S. bayanus, S. paradoxus*) and the neotype strain of *S. cerevisiae,* while relatedness between two of the synonimized species themselves is also weak, as shown here.

$$
\begin{array}{ccccc}
S.\ cerevisiae & \leftarrow 52\% \rightarrow & S.\ paradoxus \\
\uparrow & \diagdown \quad / & \uparrow \\
52\% & 20\% & 24\% \\
\downarrow & / \quad \diagdown & \downarrow \\
S.\ pastorianus & \leftarrow \mathbf{72\%} \rightarrow & S.\ bayanus
\end{array}
$$
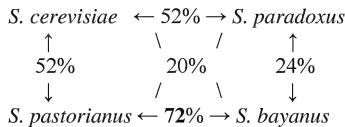
That means that *Saccharomyces cerevisiae* as a large species cannot stand. It is a group of related but distinct species: three species are shown distinct from *Saccharomyces cerevisiae sensu stricto.*

To be convincing those results needed confirmation. Kurtzman and Robnett (1991) proved the non-similarity between the three synonymized species and S. cerevisiae, using partial sequencing (300 bases) in two regions of 28S rRNA and o,e region of 18S rRNA. But, they used to charactere *S. pastorianus* the type strain of *S. carbergensis* and therefore were characterizing the later species instead.

Material and methods

The purpose of the present work is to confirm the Vaughan-Martini and Martini's statement (1987) by using the proper type strains of the same four species names: MUCL 31497, NT of *S. cerevisiae*, MUCL 31496, NT of *S. pastorianus*, MUCL 31495, T of *S. baynus*, MUCL 3149, NT of *S. paradoxus.*

rRNA extraction

Cells were grown at 25°C in 100ml of DYP liquid medium (dextrose, yeast extract, peptone) on rotary shaker at 200rpm during 16 hours. Celles were harvested by centrifugation, washed with BPS buffer and freeze dried. Undergraded rRNA was isolated according two procedures, one by Guadet *et al.* (1989) and the other by guanidinium thiocyanate (Promega). Purity of rRNA samples was estimated from spectrophotometric absorbance ratios 260/280=1.70-2.00 and their integrity by denaturating agarose gel electrophoresis (Fig. 1).
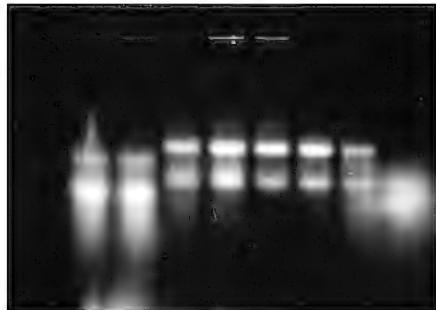
Fig. 1 Electrophoretic test of rRNA purity

rRNA sequencing

Two regions of 28 S RNA were sequenced using specific oligonucleotide primer (401), primer 5'-GGTSSGTGTTSAAGASGG-3' (635) and primer 5'-TTGGAGASSTGSTGSGG-3' (1841), totalizing 820 bases, following the dideoxynucleotide chain termination method as described by Lane *et al.* (1985). Nucleotide fragments generated in the chain elongation reactions were separated on 8% acrylamide – 8M urea gels and visualized by autoradiography. Fig. 2 presents sequences obtained from the four strains using primer 401. Alignment of the four sequences was carried out by Clustal W 1.4 program (Higgins and Sharp 1988).

Fig. 2 Sequences of 28S rRNA portion from 1, *S. pastorianus*, 2, *S. bayanus*, 3, *S. cerevisiae*, 4, *S. paradoxus*

Phylogenetic tree

A phylogenetic tree depincting the four sequenced species has been produced using the YVDP program (Van de Peer and De Wachter 1993) and including the sequences of another strain of *S. cerevisiae* and of five other fungi available from Pr De Wachter's data base, University of Antwerp. Evaluation of the branching reliability has been obtained by bootstrapping.
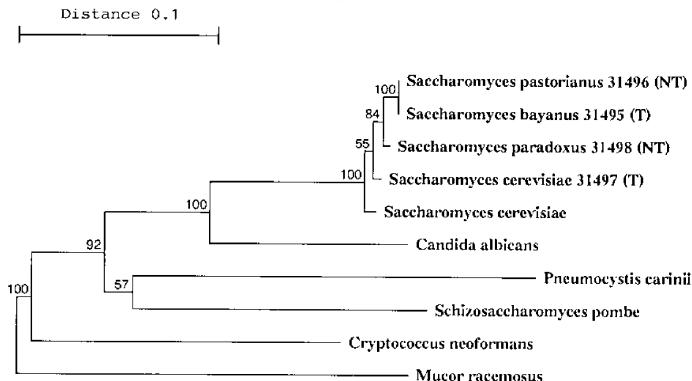
Fig. 3.Phylogenetic tree constructed using YVDP software.

Results

The obtained tree (Fig. 3) does not differ from the one obtained by Kurtzman and Robnett (1991) exepted by the fact that *Saccharomyces cerevisiae* in our tree seems to be phylogenetically older than the derivated species. This might result from sequencing a larger portion of rRNA but might also be due to the fact that the medial branching of the cluster is poorly reliable with 55% bootstrap.

In both trees the four tested *Saccharomyces* species cluster together separately from other fungal species. *Saccharomyces pastorianus* and *S. bayanus* cluster closely together with high bootstrap valuie (100%) and confirm the DNA relatedness of 72% as shown by Vaughan-Martini and Martini (1987). It also confirms the weak DNA relatedness of 20% between *S. cerevisiae* and *S. bayanus* and between *S. paradoxus* and *S. pastorianus*.

S. carlsbergensis sequenced by Kurtzman and Robnett (1985) falls into the same position than S. pastorianus. But their synonymy needs demonstration.

It is noteworthy to observe the close relatedness between *Candida albicans* and *Saccharomyces* cerevisiae, both hemiascomycetous species, and the segregation of *Pneumosystis carinii* and *Schizosaccharomyces pombe* from the Hemiascomycetes, as it has been shown by 18S rRNA sequencing. But the two latter taxa are certainly not mutually related as shown by 57% bootstrap value.

The other yeast *Cryptococcus neoformans*, is here also confirmed to be very distinct, in the same position as found in 18S rRNA trees where it culsters together with Basidiomycetes.

Conclusions

Our results based on the sequence of two portions of 28S rDNA, confirms the distinction of *Saccharomyces cerevisiae* from the three first thought synonyms *S. paradoxus*, *S. pastorianus* and *S. bayanus* and the close relatedness between *S. pastorianus* and *S. bayanus*.

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WHAT ABOUT THE "SPECIES GROUP" CONCEPT IN *ASPERGILLUS FLAVUS* ?

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Abstract. Whemer (1901) was the first to introduce the idea of "species groups" to accommodate the great diversity and variability of *Aspergilli.*

This concept is the foundation of the actual taxonomy of the genus.

The *flavus* group contains 16 species (Christensen 1985) from yellow green to brown in color. Some of them are morphologically well defined but others of industrial importance, referred as the Koji molds, *A. oryzae, A. sojae, A. tamarii* are difficult to differentiate from the toxinogenic and cosmopolitan species *A. flavus* and *A. parasiticus.*

Numerous technics have been applied to find new characters: electrophoretic mobility patterns of proteins and specific enzymes, pyrolysis profiles, DNA homology, GC contents of DNA. We propose a view of the *flavus* group through the comparison of sequences of the 5' end of 28S rRNA. Two points are clear and make the group heterogeneous:

- the complete similarity of 10 "species", including the Koji molds and the toxinogenic species,

- the split of *A. zonatus* and *A. clavatoflavus* together with the teleomorphic *Penicilliopsis.*

A COMPARATIVE STUDY OF ELECTROPHORETIC KARYOTYPES IN THE YEAST GENERA *TORULASPORA* LINDNER AND *ZYGOSACCHAROMYCES* BARKER

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Abstract. In the most recent edition of the monograph *The Yeast, a taxonomic study* (Yarrow 1984) the genera *Torulaspora* and *Zygosaccharomyces* were formally separated from *Saccharomyces*. These genera differ from *Saccharomyces* in having a predominately haploid vegetative phase and in the mechanism of ascus formation: conjugation of independent cells that can be preceded by the formation of specific protuberances or by the union of a cell with its bud.

While the distinction between *Torulaspora* and *Zygosaccharomyces* is usually straightforward, some problems have been encountered in the separation of species within *Zygosaccharomyces* since several taxa are characterized by limited assimilative and fermentative profiles. Since these genera has been clarified by DNA/DNA reassociation experiments (Price et al. 1978, Kurtzman 1991), the definition of electrophoretic karyotypes of all type strains of the two genera can be useful as an aid for the identification of unknown strains.

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FUNGAL BIODIVERITY

BIODIVERSITY OF *CYLINDROCLADIUM* ON WATER-LILIES AT SSR PAMPLEMOUSSES BOTANIC GARDEN IN MAURITIUS

A. PEERALLY

Abstract. An interesting case of fungal biodiversity within the same genus is described. Six species of *Cylindrocladium* are reported to occur on water-lilies in three contiguous ponds in Mauritius. The species are *C. hawksworthii*, recently descibed as a new species, *C. quinqueseptatum, C. colhounii, C. floridanum, C. camelliae* and *C. infestans*, all of which are pathogenic. Of these species with the exception of *C. hawksworthii*, the five others were previously observed to occur on tea in Mauritius.

Introduction

The taxonomy and pathology of the genus *Cylindrocladium* has fairly recently been comprehensively reviewed (Peerally 1991a). In the present paper the occurrence of six species of *Cylindrocladium* is reported on water-lilies at Pamplemousses Botanic Garden in Mauritius.

Three species of water-lilies, *Nelumbo nucifera* Gaertn., *Nymphaea lotus* L. and *Victoria amazonica* L. have, for many years, been known to be affected by a foliage disease (Fig. 1a-c). These water-lilies grow on three contiguous ponds, two of which contain *N. nucifera* and *N. lotus*, while the third larges pond bears *N. lotus* and the spectacular *V. amazonica*, of significant attraction to visitors. The occurrence of *Cylindrocarpon quinqueseptatum* Boedijn & Reitsma and of *C. hawksworthii* on *N. nucifera* and *N. lotus* has already been described (Peerally 1991b), since the latter pathogenen was reported as a new species.

The associatopn of *Cylindrocarpon* as a pathogen of tea had been the subject of previous papers (Peerally 1973, 1974a-C, 1976a, b, 1991a), and the species reported are:

C. colhounii Peerally
C. floridanum Sob. & Seymour
C. clavatum Hodges & May
C. camelliae Venkataaara & Venkata Ram
C. quiqueseptatum Boedijn & Reitsma
C. infestans (Boersew.) Peerally.

The occurrence of two or more species of *Cylindrocladium* on the same hosts has been frequently reported in the literature. Thus significant mortality rates of seedlings of *Eucalytus grandis* and *E. terreticornis* were due to a disease complex which included *C. quinqueseptatum, C. clavatum* and *C. ilicicola* (Anon. 1982), Mohanan and Sharma (1986) found several species of *Cylindrocladium* associated with *Eucalyptus* spp. causing a complex damping off, stem canker and leaf and shoot blight in Kerala, India.

Several papers in the literature have emphasized the disease enhancing effects of the high moisture levels with *Cylindrocladium* diseases. Thus Barnard (1984) reported extensive damage of *E. grandis* and *E. robusta* seedlings under very humid conditions caused by overhead irrigation. Various workers (Petch 1921, Loos 1951, Wabster 1954) reported that the disease incidence by *C. theae* on tea to be enhanced by wet conditions and was checked with the cessation of rain.

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Material and method

The *Cynlindrocladium* species reported in this paper were all isoltad from diseased leaves of three species of water-lilies. All six species were examined from incubated diseased material and from colonies on PDA isolated from diseased leaves after surface sterilistation. Artificially and naturally infected leaves (Fig. 2a) of water-lilies were maintained in boxes with wet cotton to induce disease and sporulation which occurred very easily after a few days at 22-27°C.

Results

The occurrence of Cylindrocladium on the three species of water-lilies wa as follows:

Host species	*Cylindrocladium* species
Nelundo nucifera	C. quinqueseptatum, C. hawksworthi
Nymphaea lotus	C. quinqueseptatum, C. hawksworthii, C. colhounii
Victoria amazonica	C. colhounii, C. floridanum, C. camelliae, C. infestans
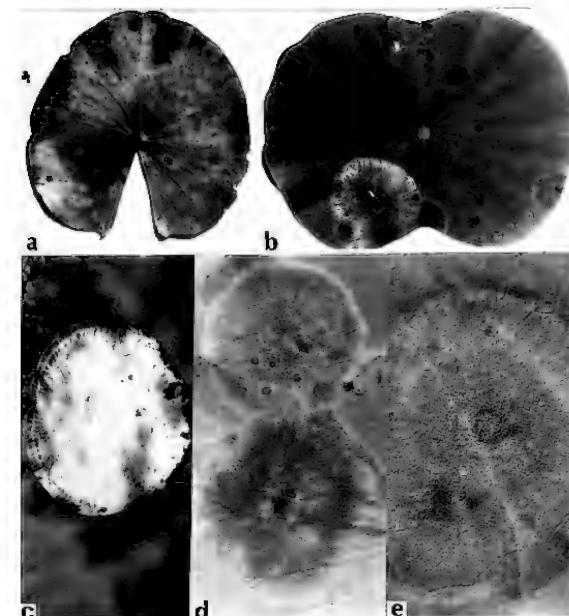
Fig. 1. a. Necrotic spots on *Nymphaea lotus* leave. b. Spot on *Nelumbo nucifera* leaf. c. Spot on *Victoria amazonica* leaf. d. *Cylindrocladium hawksworthii* (upper colony) and *C. quinqueseptatum* (lower colony) on *N. nucifera*. e. *C. hawksworthii* conidial masses on *N. nucifera*.

Spots on *Nelundo nucifera* are grey becoming brown to black and coalesce to give large circular necrotic areas. The masses of conidiophores and conidia of *C. quinqueseptatum* are generally less dense (Fig. 1d) than those of *C. hawksworthii* (Fig. 1d, e) which look decidedly denser, more powdery and white.

Spots on *Nymphaea lotus* are initially water-soaked areas turning brown and later black.

On *Victoria amazonica* (Fig. 1c) there are conspicuous water-soaked areas which soften to produce a soft rot. It can be easily observed under a microscope that in infected areas the host cells separate easily due to the dissolution of the middle lamella. Areas of infected leaves of *V. amazonica* producing condidiophoes of *C. infestans* and *C. camelliae* are clearly discernible.

Species description

Cylindrocladium colhounii **Peerally** (Fig. 2b)

Coniophores 308-413 µm long, with strile appendage, much shorter without appendage. Stipe narrower towards an apical slender vesicle 7.8-82.0 x 2.0-5.2 µm. Primary conidiophore branches aseptate, rarely 1-septate, 13-26 µm long; secondary branches aseptate, 7.8-18.2 µm long; tertiary branches aseptate 7.8-10.4 µm. Phialides in groups of 2-4, occasionally arising directly from the stipe, 7.6-13.6 µm long. Conidia hyaline, cylindrical, straight, 3-septate, rarely 1- or 2-septate, never 4- or 5-septate, 38.3-84.2 x 3.4-5.7 µm, in average 66.0-5.0 µm.

Cylindrocladium quinqueseptatum **Boedijn & Reitsma** (Fig.2c, d)

Conidiophores branches arising laterally from a stipe; primary branches aseptate or 1-septate, 8.4-26.6 µm long; secondary and tertiary branches aseptate, 11.5-15.6 µm. Phialides hyaline, 13.0-15.6 µm long. Conidia hyaline, cylindrical, straight, 1- to 6-septate, usually 5-septate, old conidia in culture developing more than 6 septa, 59.8-104.6 x 5.2-7.0 µm in vivo, 72.8-119.6 x 5.2-7.8 µm in vitro. Sterile filament terminating in a narrowly clavate vesicle 2.5-3.0 µm wide.

Cylindrocladium hawksworthii **Peerally** (fig. 3)

Conidiophores erect, hyaline, septate, 250-500 µm long. Stipe conspicuously setate; primary conidiophore branches 1-septate or aseptate 12-80 µm long; secondary branches 1-septate or aseptate 12-45 µm long; tertiary branches usually aseptate 8-30 µm long; quaterny branches usually aseptate 7-35 µm long; sterile appendage 160-280 µm; phialides solitary or in small groups, sometimes arising directly from the stipe, hyaline, aseptate, 7-13 µm long; conidia hyaline, cylindrical, curved, 1-septate, 36-62 x 3-5 µm.

Cylindrocladium floridanum **Sob. & Seymour** (Fig. 4a-d)

Conidiophores banches arising lateraly from a stipe; primary and secondary branches asteptate or 1-septate, 10.2-26 µm long; tertiary branches aseptate, 7.8-10.2 µm. Phialides hyaline, 7.8-15.6 µm. Conidia hyaline, cylindrical, 1-septate, 36.4-57.2 x 2.6-4.6 µm. Sterile filmaments temenating in a globose vesicle, 8-18 µm diam. Secondary sterile filaments present in most isolates.
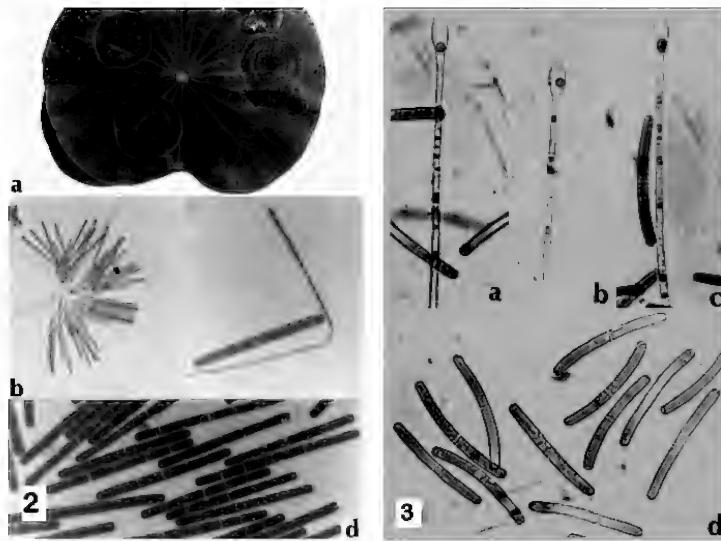
Fig.2. *Cylindrocladium colhounii.* b. Conidiophore and conidia. *Cylindrocladium quinqueseptatum.* a. spots on in vitro inoculated *Nelundo nucifera* leaf. c. Sterile appendate with vesicle. d. Conidia.
Fig. 3. *Cylindrocladium hawksworthii.* a-C. Sterile appendage with vesicle. d. Conidia.

Cylindrocladium camelliae **Venkatara & Venkata Ram** (Fig. 5c-g)

Conidiophores of two types: penicillate or verticillate. In penicillate stipe, sterile filament usually present; primary branches aseptate, 12.0-28.2 µm long; secondary branches aseptate, 8.4-19.6 µm; phialides navicular, hyaline, 9.2-18.6 µm. In verticillate stipe, sterile filament absent, phialides 16.8-31.2 µm long. Conidia hyaline cylindrical, 1-septate, 9.6-15.6 x 1.8-2.2 µm. Sterile filament elongate, aseptate, thick-walled, 60.0-110.6 µm, apically vesiculate. Vesicle lanceolate or ellipsoidal, sometimes lanceolate curved, thin walled, 4.0-5.2 µm wide. Chlamydospores present. Microconidia unfrequent in culture.

Cylindrocladium infestans **(Boesew.) Peerally** (Fig 5a, b)

Conidiophores of two types: penicillate or most often subverticillate. Penicillate conidiophores 190-250 µm long; primery branches 13-22 µm long; secondary branches 8-16 µm long; phialides avicular 5-15 µm. In verticillate conidiophores, significantly phialides longer, 14-32 µm long. Sterile filament 70-130 µm long, ending a a cylindrical or narrowly lanceolate vesicle 20-70 x 4-5 µm. Chlamydospores présent. Microconidia unfrequent in culture.

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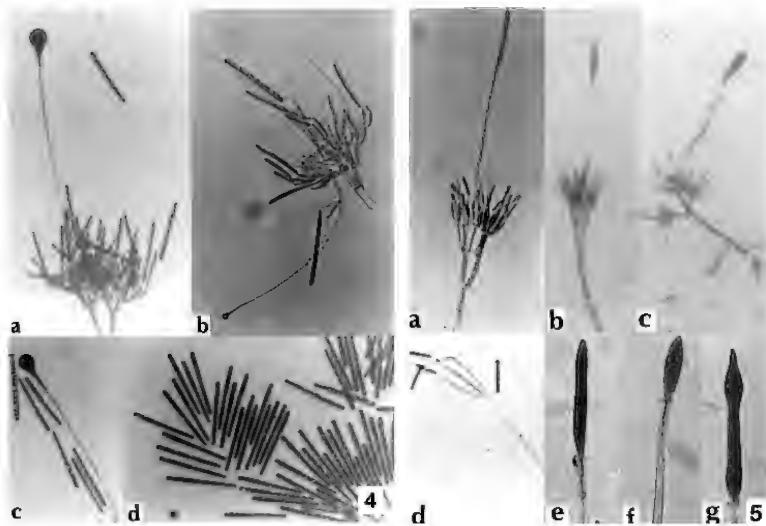
Fig. 4. *Cylindrocladium floridanum*. a, b. Conidiophores. c. Sterile appendage with vesicle. d. Conidia.

Fig. 5. *Cylindrocladium infestans*. a. Penicillate conidiophore. d. vertcillate conidiophore. *Cylindrocladium camelliae*. b-c. Penicillate conidiophores. e-g. Vesicles .

Discussion

Six species of *Cylindrocladium* pathogenic on water-lilies in Mauritius are described from three contiguous ponds in Pamplemousses Botanic Garden. In addition to the pathology of this disease complex it is interesting to highlight the biodiversity and ecological aspects. Several workers like Peerally (1974b), Rattam and Dhanda (1985) and Mohanan and Sharma (1986), have reported mixed infections involving two or morespecies of *Cylindrocladium*. The foliage disease of water-lilies in Mauritius associated with six species of *Cylindrocladium* is a particularly stricking example of the biodiversity of a single fungus genus on three species of water-lilies growing within a very small area. The fact that this disease on water-lilies occurs in a particulaly humid habitat is quite characteristic of *Cylindrocladium*, which has been constantly reported to thrive best under very humid conditions in both artificial and natural growing conditions. Of the six species occuring on water-lilies five are pathogenic to tea in Maritius in the central plateau, about 300km away from the Pamplemousses Gargen. The sixth species, *C. hawksworthii*, was described as a new species which has not so far been observed on tea or any other host.

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THE OCCURRENCE OF WOOD DECAYING FUNGI IN BELGIUM HOUSES

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Abstract. From 1990 to 1993, 602 cases of timber decay from ⬚anish⬚ houses have been analysed at MUCL. 18 species of wood decaying fungi have been recorded. 11 species cause a brown rot and represent more than 72% of the timber decay while 7 species cause white rot. *Serpula lacrymans* occurs in 46% of the cases and is the more frequent species. After this, *Donkioporia expansa*, *Coniophora marmorata* and *Asterostroma ochroleucum* are observed at a respective frequency of 11%, 7.3% and 6.3%. Noteworthy, *Coniophora marmorata* is here much more common than *Coniophora puteana*, reputed in other countries as most frequent. Another species, rarely documented as a wood decaying fungus in houses, is *Asterostroma ochroleucum*. The fungus is causing a white rot of wood and expands easily on and in plasterwork and masonry in very wet areas.
Keywords. Timber decay, house fungi, *Serpula lacrymans*, *Donkioporia expansa*, *Coniophora marmorata*, *Asterostroma ochroleucum*.

Introduction

Fungal decay in houses immediately suggests an attack by the true dry rot fungus, *Serpula lacrymans*. Indeed, *Serpula lacrymans* is the most frequently reported but also the most destructive fungi in all North Western Europe. Nevertheless, other lignicolous basidiomycetes occur in buildings beside *Serpula lacrymans*. For instance *Coniophora puteana*, the cellar fungus, is known as frequent. But few informations are available over the presence and frequency of others species than the true dry rot in buildings of central and western Europe. Koch (1985) gives an overview of the principal wood-rot fungi occurring in Danish buildings for years 1946-1983. More recently, Paajanen and Viitanen (1989) do the same for Finland for the years 1978 to 1988.

From 1990 to 1993, 683 cases of timber decaying fungi were analysed by MUCL. This paper presents a brief draft of the species encountered and their frequency in Belgian housing with some comments on noteworthy species.

Situation in Belgium

Between 1990 and 1993, 683 timber decaying fungi were analysed at MUCL. Most samples were sent or brought to the laboratory by private owners or repairing companies. 92.4% of the samples were identified to the species level, doubtful cases identified only to the genus level, representing 7.6%. 18 species belonging to 8 families have been recorded. Table 1 gives the list of species encountered, their respective case number and frequency. Table 2 gives the families with their respective species number, incidence and kind of rot (classification according to Jülich 1981). 11 species cause a brown rot -or laccase negative rot- while 7 species cause a white rot -or laccase positive rot. Incidence of brown rotter is much more important than that of white rotter. Indeed, more than 72% of timber decay is by brown rot against only 28% by white rot. Almost two third (62.7%) of the decay are assigned to members of the *Coniophoraceae* represented by 3 genera and 5 species.
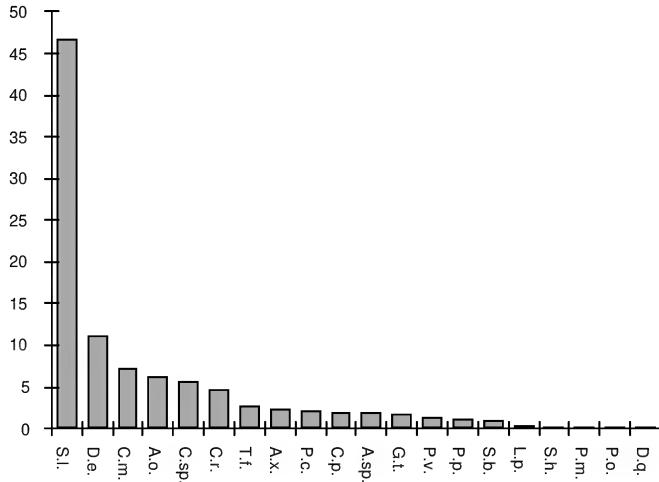
S.l.: *Serpula lacrymans* – D.e.: *Donkioporia expansa* – C.m.: *Coniophora marmorata* – A.o.: *Asterostroma ochroleucum* – C. sp.: *Coniophora species* – C.r.: *Coprinus radians* – T.f.: *Trechispora farinacea* – P.c.: *Phellinus contiguus* – A.x.: *Antrodia xantha* – C.p.: *Coniophora puteana* – A.sp.: *Antrodia species* – G.t.: *Gloeophyllum trabeum* – P.p.: *Paxillus panuoides* – P.v.: *Poria vaillantii* – S.b.: *Sistotrema brinkmanii* – L.p. *Leucogyrophana pinastri* – S.h.: *Serpula himantioides* – P.m.: *Perenniporia medulla-panis* – P.o.: *Pleurotus ostreatus* – D.q.: *Daedelea quercina*.

Table 1. List of species seen during the studies and their respective number, frequency and kind of rot.

Species	raw number	Percentage	Kind of rot
Serpula lacrymans	319	46.7	Brown
Donkioporia expansa	76	11.12	White
Coniophora marmorata	50	7.32	Brown
Asterostroma ochroleucum	43	6.29	White
Coniophora sp.	39	5.7	Brown
Coprinus radians	32	4.68	White
Trechispora farinacea	19	2.78	White
Antrodia xantha	16	2.34	Brown
Phellinus contiguus	15	2.19	White
Coniophora puteana	14	2.05	Brown
Antrodia sp.	13	1.90	Brown
Gloeophyllum trabeum	12	1.75	Brown
Antrodia vaillantii	10	1.46	Brown
Paxillus panuoides	8	1.17	Brown
Sistotrema brinkmanii	7	1.02	Brown
Leucogyrophana pinastri	3	0.44	Brown
Serpula himantioides	2	0.30	Brown
Perenniporia medulla-panis	2	0.30	White
Pleurotus ostreatus	2	0.30	White
Daedalea quercina	1	0.15	Brown
TOTAL	683	100	

Table 2. List of families encountered with their respective frequency and kind of rot.

Fungal family	Brown rot		white rot		Total	
	species	% incidence	species	% incidence	species	% incidence
Coniophoraceae	5	62.5 (427)	-	0 (0)	5	62.5 (427)
Polyporaceae	4	7.6 (52)	2	11.4 (78)	6	19 (130)
Lachnocladiaceae	-	0 (0)	1	6.3 (43)	1	6.3 (43)
Coprinaceae	0	0 (0)	1	4.7 (32)	1	4.7 (32)
Corticiaceae	1	1.0 (7)	1	2.8 (19)	2	3.8 (26)
Hymenochaetaceae	-	0 (0)	1	2.2 (15)	1	2.2 (15)
Paxillaceae	1	1.2 (8)	-	0 (0)	1	1.2 (8)
Pleurotaceae	-	0 (0)	1	0.3 (1)	1	0.3 (1)
Total number of species	11	72.3 (493)	7	27.7 (190)	18	100
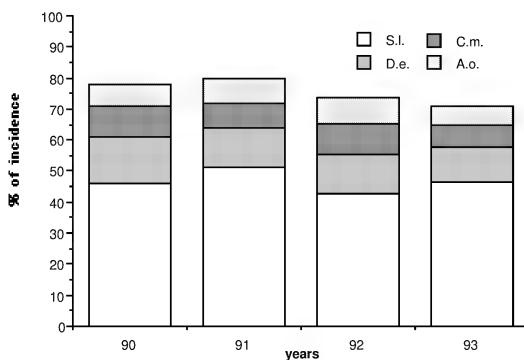
Fig. 2. Frequency of the 4 principal species year by year.

Discussion

Before discussing the present data, we must advice that relative occurrence given above are not necessarily representative of the real Belgian situation. In first hand, we do not have information from all around Belgium. Most of the specimens analysed originate from Brussels and Louvain-la-Neuve areas. In second hand, we have not collected all the samples ourselves in a randomly way. Most were sent to MUCL and we can not certify they were always collected randomly. It may occur that a selection has been applied before. This is particularly obvious for *Serpula lacrymans*. Indeed, information about the dry rot in house has been widely spread in the public the last years in Belgium by means of media, books and vulgarisation leaflets (Leclercq 1989, Rammeloo *et al.* 1989, 1992, Hennebert *et al.* 1990). Peoples dealing with timber decay (owners, repair companies, architects and insurance experts) may have acquired by this way a relatively good knowledge of the true dry rot symptoms allowing them to identify *Serpula lacrymans*. Samples sent to the laboratory might be only the most critical ones. This can alter the real species occurrence, leading to some under-estimation of *Serpula lacrymans* and consequently to some over-estimation of the others species.
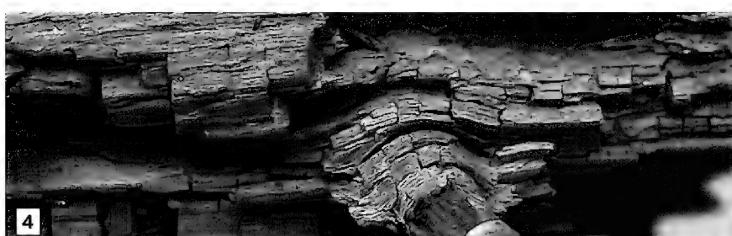
Fig.3. Cubic rot or Brown rot due to *Serpula lacrymans*.
Fig.4. Micro-cubic rot due to *Coniophora puteana*.
Fig.5. Fibrous rot or White rot due to *Donkioporia expansa*.

The occurrence given above must therefore be regarded more as a general overview rather than the real situation which could be slightly different. Nevertheless, for the other species than *S. lacrymans*, the number of cases per year represents a good draft of the situation. More than the real occurrence, the presence and the relative position of each species are important.

1. *Serpula lacrymans* (Wulfen:Fries) Schroeter

Serpula lacrymans, the true dry rot fungus, as in all north western European countries, is by far the most important species involved in timber decay. Almost 50% of the timber breakdown is attributed to that species. It is also the most destructive fungi for timber.
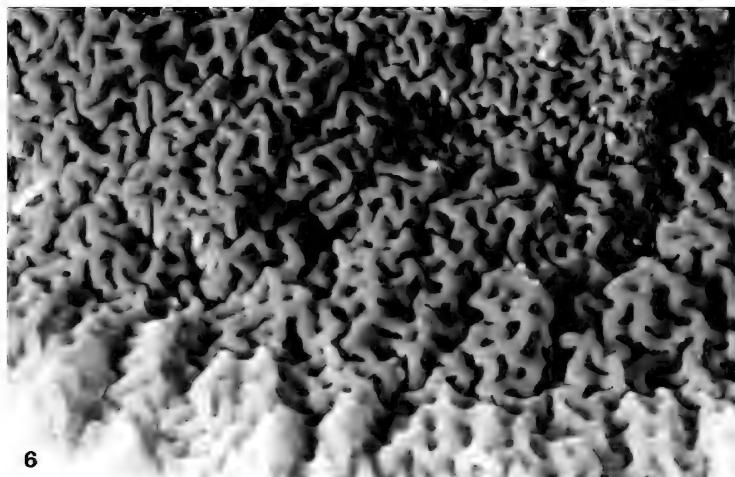
Fig. 6. Hymenium of the fruitbody of *Serpula lacrymans*.

2. *Donkioporia expansa* (Desmazières) Kotlaba & Pouzar

Syn. *Boletus expansus* Desmazières
   Poria expansa (Desmazières) H. Jahn
  Phellinus cryptarum Karsten
  Polyporus megaloporus Persoon
   Phellinus megaloporus (Persoon) Heim
   Poria megalopora (Persoon) Cooke

Donkioporia expansa (Desmazières) Kotlaba & Pouzar comes in second position with an occurrence of 11% (76 cases in 4 years). This species causes a white rot mainly of deciduous wood but can also be found on coniferous (Cartwright and Findlay 1958, Findlay 1950, Buchwald 1986).

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According to Heineman (1979), the earliest Belgian record of this species dates on 1854. This author considered this species rare in Belgium and cited only 5 more records after the first one (Heineman 1979). Since then, in 1989, Guillitte (in Rammeloo *et al.* 1989) noted a very weak occurrence of only 2 % (5 cases over 250 samples between 1985 and 1988). In 1992, the same author (in Rammeloo *et al.* 1992) mentioned 71 cases from 1985 to 1991 (for 749 wood decaying fungi analysed in 7 years), thus ± 10 cases/year. In the present analysis one the 4 last years, 76 cases have been identified with an average of 19 cases/year

D. expansa was also considered as scarce in others western European countries. The species is known in Belgium since at least 1922 (as *P. cryptarum*) after the destruction of the Versailles Palace woodwork (Mangin and Patouillard 1922). There is no recent data available on the occurrence of the species in Belgium. In England, Findlay (1950) reports the species in «a dozen or more buildings». Jülich (1984) reports the same as rare in CS, D, DDR, GB and NL. Ritter (1983) draws attention on the species in a small report. As far as we know, Buchwald (1986) was the first to demonstrate that *D. expansa* occurs much more frequently in Germany than previously known. He reported an average of 15 to 20 cases/year while the species was stated as seldom.

Nor Koch (1985) nor Paajanen and Viitanen (1989) do mention the species in their survey of timber decaying fungi in North Europe. According to Ryvarden and Gilbertson (1993) *D. expansa* is a Central and Southern species.

Buchwald (1986) draws the attention on the fact that *D. expansa* is not strictly restricted to hardwood (oak and chestnut mainly) as stated previously but is also able to decay softwood. Well, in the past, most records were made on oak or chestnut wood (Cartwright and Findlay 1958, Findlay 1950), but it is well demonstrated now that th species occurs also on coniferous wood. Numerous collections are made on softwood now since oak wood is less used in modern buildings.

For more literature concerning this species, see Mangin and Patouillard 1922 (under *P. cryptarum*), Cartwright and Findlay 1958 (under *P. cryptarum*), Domanski and Orlicz 1967 (under *Polyporus megaloporus* Persoon), Dörfelt and Sommer 1973 (under *Poria expansa*), Heineman 1979 (under *D. expansa*), Ritter 1983 (under *D. expansa*), Buchwald 1986 (under *D. expansa*),

3. *Coniophora marmorata* Desmazières.

Coniophora marmorata Desmazières represent 7.9% of the specimens analysed (40 cases over 520) while *Coniophora puteana* represent only 2.4% (12 cases). Up to 1992, no mention of this species existed for Belgium. Ginns (1982), in its monograph of the genus *Coniophora* cited only 9 specimens. Few data on its occurrence in Europe are available. *C. marmorata* seems to be restricted to western, central and southern Europe. Indeed, nor Koch (1985) nor Paajanen and Viitanen (1989) mention the species in their survey. At the same, Hallenberg (1985) do not mention the species for northern Europe. However Beech-Andersen (1992) mentions the species in Denmark.
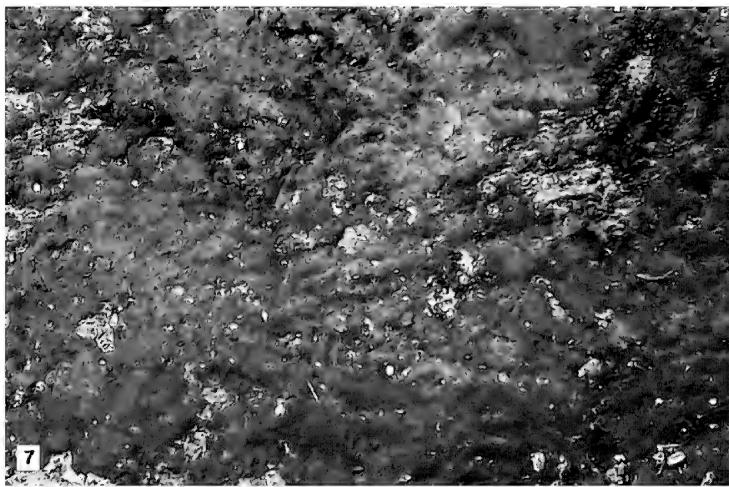
Fig. 7. fruitbody of *Coniophora marmorata* on wood.

Table 2. Main distinguishing features between *C. marmorata* and *C. puteana*(Ginns, 1982).

Speciies	Spores	Mycelium
C. marmorata	subglobose to ovoid dextrinoid 7-10 µm x 4.5-7 (-8) µm	dimitic with tan to pale yellow skeletal hyphae 2-7 µm wide
C. puteana	broadly ellipsoid non dextrinoid 9.5-14 x 6-7.5 µm	monomitic

4. *Asterostroma ochroleucum* **Bresadola.**

Asterostroma ochroleucum Bresadola represents 6.2% of samples analysed (43 cases over 683).

A. ochroleucum produces a white fibrous rot of both hardwood and coniferous wood. It expands easily on and in plasterwork and masonry in very wet areas forming whithish to ochraceous strands network which can be mistaken for *S. lacrymans* strands with the naked eyes. It is easily recognised under the microscope by its typical brown asterosetae. When fruitbodies is present, which occurs scarcely in Belgian buildings, spores are globose to subglobose and strongly tuberculate (fig. 9) with amyloid tubercule.

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Fig.8. Mycelium et fruitbody (in centre) of *Asterostroma ochroleuca* on plasterwork.

Fig.9. Asterosetae and tuberculate spores of *Asterostroma ochroleucum.*

Few data are available on the occurrence of the species in building, in Europe. Findlay (1950) found the species on several occasions in England while Koch, according to Hallenberg (1985), mentioned it as quite common in Denmark.

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However, she did not mention it in her survey of wood decay fungi in Danish houses (Koch 1985).

This species is more important than previously known.

Conclusions

Like in all North and Western European countries, *Serpula lacrymans* is the first cause of wood decay in Belgium. It is also the most destructive fungus. From the data analysed, the species is involved in almost 50 % of the wood damage in Belgium. And its occurrence is most probably under-estimated.

Donkioporia expansa is much more important than previously established. It has to be looked for, not only on oak wood but also on coniferous wood.

Coniophora marmorata is also an important wood decayer and has been certainly mistaken for *C. puteana* in the past.

Asterostroma ochroleucum is not uncommon in Belgian buildings and was certainly overlooked till recently..

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SYMBIOTIC FUNGI IN THE GALLERIES OF THE STRIPED BARK BEETLE, *TRYPODENDRON LINEATUM* (COLEOPTERA, SCOLYTIDAE)

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Abstract. Symbiotic fungi from the galleries of the striped bark beetle, *Trypodendron lineatum* (Ol.) found in spruce logs lying at the timber storage yard of a woodprocessing industry in Ljubljana were studied. In laboratory conditions the following fungi were isolated and determined: *Trichoderma harzianum* Rifai, yeast *Pichia anomala* Hansen (Kurzman), blue-stain fungus *Ceratocystis piceae* (Münch) Bakshi (anam. *Graphium*) and a fungus closely similar to *Ceratocystis araucariae* (anamorph) (in the following text designed as *FsCa*). Isolated fungi stained the wood but did not cause any major weight loss of wood samples in laboratory conditions. The hyphae of symbiotic fungi primarily colonized the parenchyma tissues of the sapwood, and also tracheids. Enzyme activity of fungi was studied by using simple laboratory test methods. The presence of specific enzymes cooperating in wood decay was established.

Introduction

The striped bark beetle, *Trypodendron lineatum* (Ol.) is considered to be one of the most serious pest among ambrosia beetles in the northern hemisphere (Uusvaara and Loyttyniemi 1975). Ambrosia beetles are coleopteran insects (Scolytidae and Platypodidae) that indirectly utilize wood.

The damage is caused by the female beetle of *T. lineatum* tunneling in sapwood and introducing into it spores of mutualistic fungi which then grow in the gallery. Such fungi can spread and stain the wood near gallery or up to several centimeters from the gallery. They convert wood components into the fungal growth which serves as food for the beetle (Baker, 1963; Batra, 1963). *T. lineatum* attacks logs of autumn and winter felled coniferous trees lying unpeeled, and also weakened or dying trees (Anilla, 1975).

From the first reports of symbiosis between insects and fungi till the early 1960s, the concept of one ambrosial fungus per one ambrosia beetle was emphasized. But some subsequent studies confirmed the view that each ambrosia beetle is symbiotically associated with more than one fungus (Baker 1963, Norris 1965). Experimental results show that different species of bacteria, yeasts, yeastlike fungi and ambrosial fungi compose the symbiotic community of microorganisms with the ambrosia beetle. Norris (1979) used for the assemblage of symbiotic micro- and macroorganisms (including the beetle) the term a multi-species complex or a supraspecies. The activities of the microbial complex as a whole, not just those of the ambrosial fungus, allow such beetles to indirectly utilize nutrient-poor substrates as wood.

Nevertheless, many questions concerning the relationship and interaction between symbiotic fungi and ambrosia beetles are still open. The following problems are perhaps the most interesting for research in near future: isolation and determination of fungi species associated with the ambrosia beetles in different countries, antagonism and synergism among symbiotic fungi *in vivo* and *in vitro*, the influence of fungi on wood decay and utilization of wood components, succession of fungi in attacked wood etc.

Materials and Methods

Isolation of fungi

Spruce logs containing galleries of active *T. lineatum* were identified by the presence of the whitish bore-dust at the entrance holes in bark crevices and by chiseling into galleries. 20cm-thick disks of the logs were removed in areas containing the highest number of the entrance holes. These disks were then brought to laboratory for dissection and isolation of associated fungi.

Isolations were made from the different parts of the galleries: entrance tunnels, lateral mother tunnels, axial larval cradles, wood near tunnels and abandoned tunnels. Samples were taken with sterile forceps and scalpel. Each sample was brought on the surface of potato-dextrose agar (PDA).

Adult females were aseptically removed from the tunnels and allowed to walk on agar surfaces, to transfer the spores of fungi to the medium.

Determination of decay capacity

Decay capacity of isolated fungi was determined by partly modified EN 113. In laboratory conditions the wood samples of spruce (*P. abies* (L.) Karst.) and red pine (*P. sylvestris* L.) were exposed to fungi activity on malt extract agar (MEA) in Kolle flasks for 16 weeks. The decay of wood samples was determined by measuring weight loss.

Enzyme activity

The presence of specific enzymes and their activity in the symbiotic fungi were demonstrated by simple test methods. Cellulase activity was determined according to Rautela and Cowling (1966), that of oxidase according to Bavendamm (1928) and Jorgensen and Vejlby (1953). Activity of laccase, which is important for the splitting of the phenol ring of the lignin complex, was determined according to Stalpers (1978).

Results and discussion

From the different parts of the galleries of *T. lineatum,* four species of symbiotic fungi were constantly isolated: *Trichoderma harzianum* Rifai, yeast *Pichia anomala* (Hansen) Kurzman, blue-stain fungus *Ceratocystis piceae* (Münch) Bakshi (anam. *Graphium*) and a fungus closely similar to *Ceratocystis araucariae* (anam. *'FsCa'*) (Table 1).

The ambrosial fungus of *T. lineatum* known as *Ambrosiella ferruginea* (Mathiesen-Käärik) Batra was not isolated. The four species of fungi were found during the pupa, callow adult and adult beetle stage. Some other microorganisms were isolated from either the beetles or their galleries, but not from both. They were found at only one, or a very few locations on beetles or in galleries. Therefore, they were not considered as symbionts and not determined.

Nevertheless, no report on the establishment of *T. harzianum*, *P. anomale* and *FsCa* from the galleries of *T. lineatum* were found in the literature. Consequently, this might be the first observation of the symbiosis of *T. lineatum* with these fungi. It is known for a long time that *C. piceae* is an important member of the fungal community of microbes associated with different ambrosia and bark beetles (Bakshi

1950, Wilson 1959, Käärik 1971). Because of limited and extremely slow growth *P anomala* was not used in further experiments.

Table 1: Symbiotic fungi isolated from the different parts of the galleries of *Trypodendron lineatum* (Ol.).

Symbiont	Entrance tunne	Lateral mother tu	Axial larval cr	Wood near	Abandoned tunnel
T. harzianum	+	+	+	+	+
P. anomala	+	+	+	-	-
C. piceae	+	+	+	+	+
FsCa	+	+	+	+	+

The symbiotic fungi caused the stain of spruce and red pine wood after 16 weeks, but did not produce any major weight loss of wood samples. Weight loss was found to be the highest in samples exposed to *C. piceae*, and medium weight loss in samples exposed to *FsCa* which caused the most intensive wood staining. The lowest weight loss was observed in samples exposed to *T. harzianum* which discoloured the wood by forming masses of pigmented spores on the wood surface (Table 2). *C. piceae* caused 11.4 % weight loss in American beech and 17.3 % in sugar maple after 3 months (Eslyn and Davidson, 1976). Fungus can act as typical soft-rot and attack the S2 layer of tracheid walls (Levy, 1967).

Table 2: Weight loss of wood samples (%) produced by symbiotic fungi of *Trypodendron lineatum* (Ol.).

Symbiont	cellulase	oxidase	laccase
T. harzianum	+	-	
C. piceae	++	++	+++
FsCa	+++	-	+++

a) mean values of weight loss of ten samples; b) standard deviation

Specific enzymes are needed for the fungi to penetrate the wood elements. Cellulase was proved in *T. harzianum, C. piceae* and *FsCa*. Laccase activity was observed in *FsCa* and *C. piceae* which produced also oxidase.*T. harzianum* did not synthesize any of these enzymes (Table 3).

Table 3: Enzyme activity of symbiotic fungi of *Trypodendron lineatum* (Ol.). - = no activity; + = slight

Symbiont	Red pine		Spruce	
	$\overline{X}$[a]	SD[b]	$\overline{X}$[a]	SD[b]
T. harzianum	0.05	0.002	0.07	0.002
C. piceae	2.21	0.012	1.02	0.009
FsCa	0.79	0.012	0.12	0.006

activity: ++ = medium activity; +++ = strong activity.

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CERCOSPOROID FUNGI ASSOCIATED WITH THE EYESPOT DISEASE OF GRAMINICOLOUS HOSTS

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Introduction

The fungus commonly associated with eyespot disease symptoms of wheat was first described as *Cercosporella herpotrichoides* Fron (Sprague 1936). Based on the unthickened, inconspicuous conidial scars, several *Cercosporella* species were reallocated to the newly erected genus *Pseudocercosporella* Deighton (1973). Two varieties of *P. herpotrichoide*s (Fron.) Deighton were distinguished, namely *P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis* Nirenberg (Nirenberg 1981). In addition, *P. anguioides* Nirenberg and *P. aestiva* Nirenberg were also described from eyespot symptoms on cereals in Germany (Nirenberg 1981). In a study of the various *Mycosphaerella* Johanson species and their respective anamorphs (von Arx 1983), *P. herpotrichoides* was reallocated to the genus *Ramulispora* Miura as *R. herpotrichoides* (Fron) Arx . This decision was chiefly based on the fact that *Ramulispora* species had conidia forming lateral branches. The genus was furthermore restricted to all species occurring on Poaceae. *Pseudocercosporella* species were recognized as anamorphs of *Mycosphaerella* (Corlett 1991). The description of the teleomorph of *Ramulispora herpotrichoides* as *Tapesia yallundae* Wallwork & Spooner (1988) [*Oculimacula yallundae* (Wallroth & Spooner) Crous & W. Gams, 2003, ndlr], further supported its separation from *Pseudocercosporella*.

New combinations for the varieties of *R. herpotrichoides* were subsequently introduced as *R. herpotrichoides* var. *herpotrichoides* (Fig. 1) and var. *acuformis* (Nirenberg) Boerema, Pieters & Hamers (1992). This variety was later reconsidered at species level as *R. acuformis* (Nirenberg) Crous. Braun (1993) furthermore reduced *Pseudocercosporella. anguioides* to an additional variety of *R. herpotrichoides*. No reasons were given, however, for not retaining it as a separate species in *Ramumispora*. Because of the uncertainty surrounding the generic placement of *Pseudocercosporella aestiva* (Fig. 2), the latter species was not introduced into *Ramulispora*.

Using isozyme and mitochondrial DNA (DNA) banding patterns, the three varieties of *Ramulispora herpotrichoides* could clearly be distinguished from *P. aestiva* (Julian & Lucas 1990, Priestly *et al.* 1992; Nicholson *et al.* 1993), which appeared to be unrelated to *R. herpotrichoides*. The aim of the present study, therefore, was to use scanning electron microscopy (SEM) and random amplified polymorphic DNA (RAPDs) to investigate the generic and species status of the four taxa discussed above.

Material and Methods

Scanning electron microscopy

Verified isolates of *R. herpotrichoides* var. *herpotrichoides* (CBS 494.80), var. *acuformis* (CBS 495.80), var. *anguioides* (86.45.64C) and *P. aestiva* (CBS 497.80) were used to compare their conidial morphology using SEM. Sporulation of isolates were obtained after 2 wks on synthetic nutrient agar (SNA) (Nirenberg 1981) at

15°C. Specimens for SEM were cut from colonized agar, fixed overnight in 3% glutaraldehyde, and rinsed in 0.05 M Na Cacodylate buffer the next day. The material was further fixed in 2% Osmium tetroxide in 0.05 M Na Cacodylate buffer, rinsed in the latter buffer, and dehydrated in a series of alcohol concentrations. Specimens were critical point dried, coated with gold and viewed with a Jeol JSM 6100 scanning electron microscope. Specimens were also viewed using kryo-fixation.

DNA isolation

Colonized agar plugs from 14-d-old colonies grown on PDA were inoculated into 200 ml liquid cultures (8 g/l yeast extract and 5 g/l glucose). Cultures were incubated at 25°C for 10 d on an orbital shaker and the mycelia harvested by filtration (Whatman no.1 filter paper). Freeze-dried mycelia mixed with river sand was ground to a fine powder with a mortar and pestle. Twenty ml lysis buffer (3% [v/v] SDS; 1% [v/v] 2-mercaptoethanol; 50 mM Tris [pH 7.2] and 50 mM EDTA) was added and the mixture incubated at 65°C overnight. Cetyltrimethylammonium bromide (CTAB) and 5 M NaCl were then added to a final concentration of 1% [v/v] and 0.7 M respectively and incubated for 4 hrs at 65°C. The mixture was extracted with phenol/chloroform/isoamylalcohol (25:24:1) (PCI), and followed by two extractions with chloroform/isoamylalcohol. The resulting aqueous fraction was mixed with 7.5M NH4OAc and 0.54 volumes isopropanol and incubated at -20°C overnight to precipitate the nucleic acids. After centrifugation at 5000 rpm for 5 min, the pellet was washed with 70% [v/v] ethanol, vacuum dried and dissolved in 300 µl TE buffer (10 mM Tris [pH 8.0] and 1 mM EDTA). The sample was treated with Rnase A (10 mg/ml) and incubated at 37°C for 90 min after which the DNA was PCI extracted, precipitated with isopropanol in the presence of NH4Oac, washed, vacuum dried and redissolved in 300 µl TE buffer.

RAPD analysis

The 10-mers used as random primers were purchased from Operon Technologies (Kit E, Operon Technologies Inc. Alameda CA94501, USA). Taq Polymerase together with its 10X concentrated buffer (100 mM Tris-HCl [pH 8.3] [20°C]; 15 mM MgCl2; 500 mM KCl) was supplied by Boehringer Mannheim (Boehringer Mannheim, Johannesburg, South Africa). PCR reaction mixtures (50 µl final volumes) contained 25 ng genomic DNA, dATP, dCTP, dGTP and dTTP each at 200 µM final concentration, 100 nM oligonucleotide primer, 1X Taq polymerase buffer and 1 unit of Taq polymerase. One mM MgCl2 was also added. Each reaction was overlaid with a 100 µl of mineral oil to prevent evaporation. The random sequence primers used were OPE-14 5'TGCGGCTGAG and OPE-15 5'ACGCACAACC. Amplifications were conducted in a Hybaid Omnigene (Hybaid Ltd., Waldegrave Rd., Middlesex, UK) thermal cycler. Samples were subjected to 60 repeats of the following cycle: 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. After the last cycle, a final extension step of 72°C for 5 min was included, followed by cooling to 4°C until recovery of the samples. Electrophoresis of aliquots (15 µl) of amplification products was through 1.4% agarose gels using 1X TAE buffer. Phage lamda DNA digested with EcoRI and HindIII was used as a standard.

Results and Discussion

Generic placement

When von Arx (1983) separated the graminicolous *Pseudocercosporella* species into *Ramulispora*, it was chiefly based on the hosts falling in the Poaceae, and the conidia having the ability to form lateral branches.

All species of *Pseudocercosporella* studied by the present authors have been found to have conidia arranged in dry masses. However, conidia of *R. sorghi* (Ell. & Ev.) Olive & Lefebvre, the type species of *Ramulispora*, aggregate in slimy masses. The same phenomenon has also been observed for *R. herpotrichoides*.

With the description of the teleomorph of *R. herpotrichoides* var. *herpotrichoides* in *Tapesia*, additional support was given for the separation of these anamorphs from *Pseudocercosporella*. Furthermore, based on the research of King (1990), *T. yallundae* var. *acuformis* Boerema, R. Pieters & Hamers (1992) has also recently been described as the teleomorph for *R. herpotrichoides* var. *acuformis*.and later named *Tapesia acuformis* [*Oculimacula acuformis* (Boerema, R. Pieters & Hamers) Crous & W. Gams, 2003; ndlr].

Other than *Tapesia* and *Mycosphaerella* teleomorphs, the formation of lateral conidium branches is the chief morphological character proposed by von Arx (1983) to separate *Pseudocercosporella* from *Ramulispora*. Von Arx (1983) clearly illustrated lateral branches in *Ramulispora sorghi* and *R. herpotrichoides* as is typically observed in other Cercosporoid genera such as *Mycovellosiella* Rangel (Crous & Braun 1994). However, in a paper dealing with the morphology of *R. sorghi*, Olive *et al.* (1946) presented several figures indicating that the lateral branches in *R. sorghi* are in fact secondary conidia forming directly on the surface of primary conidia. Chang and Tyler (1964) were the first to report microcyclic conidiation in *R. herpotrichoides* var. *herpotrichoides*. Fernandez et al. (1991) recently defined this process of conidiation in *Cercospora kikuchii* (Matsumoto & Tomoyasu) M.W. Gardner. Although Chang and Tyler (1964) reported short secondary conidiophores to be present, the latter was not observed in our study. Secondary conidia were, however, found to develop directly on the surface of primary conidia. Microcyclic conidiation, also known as iterative germination or precocious sporulation was defined as the recapitulation of conidiation following conidial germination without an intervening phase of mycelial growth.

The phenomenon observed for *R. herpotrichoides* and *P. aestiva* in the present study was identical to that illustrated by Olive *et al.* (1946) for *R. sorghi*, and distinct from microcyclic conidiation (sensu Fernandez *et al.* 1991), and lateral branches (sensu Crous & Braun 1994). We conclude, therefore, that in *Ramulispora* the formation of secondary conidia and not lateral branching or microcyclic conidiation occurs. The process should therefore be seen as conidia forming directly on the surface of the primary conidia, without the presence of an intervening germ tube with conidiogenous locus.

From these data it is clearly shown that the three varieties discussed above are presently best retained in *Ramulispora*, as they cannot be accommodated in *Pseudocercosporella*. The generic placement of *P. aestiva* remains, however, uncertain. The present study suggests that the possible collection of its teleomorph and further molecular studies will prove necessary to resolve the generic position of *P. aestiva*.

Species delimitation

Ramulispora herpotrichoides **var.** *herpotrichoides* **& var.** *acuformis*

Several morphological criteria are used to distinguish var. *herpotrichoides* and var. *acuformis*. Conidia of var. *herpotrichoides* are curved, whereas those of var. *acuformis* are straight (Nirenberg 1981). Our SEM study showed both var. *herpotrichoides* and var. *acuformis* to have polyblastic conidiogenous cells (Fig. 3). When cultured on PDA, var. *herpotrichoides* has fast growing colonies with smooth margins, whereas *R. herpotrichoides* var. *acuformis* has slow growing colonies with feathery margins (Lange-de la Camp 1966, Scott *et al.* 1975). The two varieties also differ in their pathogenicity towards wheat and rye, with var. *herpotrichoides* being more pathogenic to wheat than to rye, and var. *acuformis* being equally pathogenic to wheat and rye (Lange-de la Camp 1966, Hollins *et al.* 1985). Furthermore, an ultrastuctural study showed that the infection process on a wheat coleoptile was also distinctly different for var. *herpotrichoides* and var. *acuformis* (Daniels *et al.* 1991). Distinct mating populations have also recently been isolated for var. *acuformis* and var. *herpotrichoides* in England (P. Dyer, personal communication). Molecular markers including isozymes, restriction fragment length polymorphisms (RFLPs) and RAPDs have successfully been used to distinguish var. *herpotrichoide*s and *var. acuformis* (Julian and Lucas, 1990; Nicholson *et al.* 1991, 1993, Nicholson and Rezanoor 1994, Priestley *et al.* 1992, Thomas *et al.* 1992).

Using RAPDs, the present study supported the distinction made between var. *herpotrichoides* and var. *acuformis* based on general morphology, cultural characteristics, pathology, and molecular studies of other workers discussed above. The F-values obtained with two primers in the present study indicated var. *herpotrichoides* to have 50% similarity to var. *acuformis*. Using four primers, this figure decreased to 35% (Fig. 5, Table 1). This low percentage similarity, together with the other differences discussed above, suggests that these two varieties should be seen as two separate species.

Ramulispora herpotrichoides **var.** *anguioides*

Nirenberg (1981) described var. *anguioides* as having grey- pink, velvety colonies on PDA with even colony margins. Conidia were described as forming on simple, seldomly branched conidiophores. Successful protoplast fusion between var. *herpotrichoides,* var. *acuformis* and var. *anguioides* has recently been obtained by Hocart & McNaughton (1994), indicating that these varieties are closely related.

Using isozyme electromorphs, var. *anguioides* has been shown to be distinct from var. *herpotrichoides,* var. *acuformis* and *P. aestiva* (Julian and Lucas 1990, Priestley *et al.* 1992). However, var. anguioides has been shown to have a certain percentage of similarity with var. acuformis. Using RFLPs, Nicholson et al. (1993) found that strong hybridization occurs between var. *anguioides* and var. *herpotrichoides*, indicating that they may be closely related. However, DNA electromorphs of the three varieties were distinct.

Using RAPDs, the present study found var. anguioides to be distinct from the other two varieties and *P. aestiva*. F-values showed var. *anguioides* to have 17%, 9% and 8% similarity to var. *herpotrichoides*, var. *acuformis* and *P. aestiva* respectively (Fig. 5, Table 1).

Pseudocercosporella aestiva:

Nirenberg (1981) described the conidiophores of var. *anguioides* as hyaline, thin, polyblastic, simple, and seldomly branched. Results obtained in the present study found all four taxa could have polyblastic conidiogenous cells. However, the conidiogenous cells of var. *herpotrichoides* were frequently swollen and ampulliform, whereas those of *P. aestiva* were mostly reduced (Fig. 4).

Isozyme and RFLPs have indicated little relationship between *P. aestiva* and the three varieties of *R. herpotrichoides* (Julian and Lucas 1990, Nicholson *et al.* 1993, Priestley *et al.* 1992).
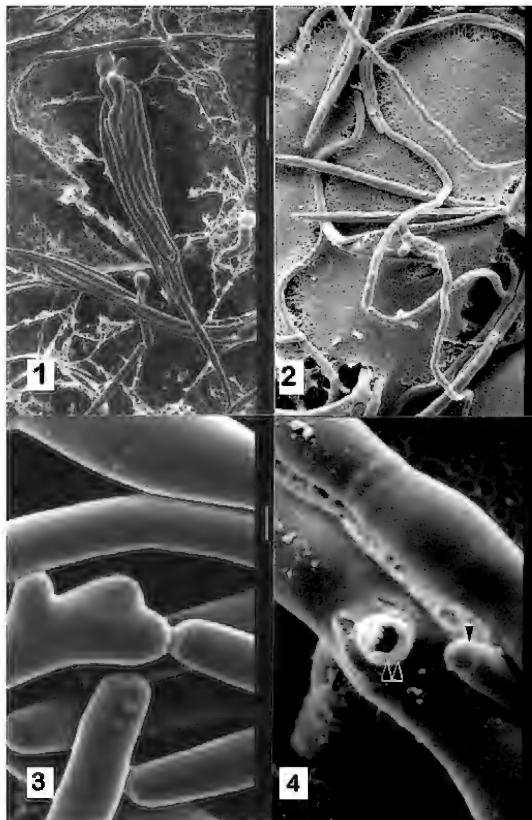
Fig.1. Conidiophore of *R. herpotrichoides* var. *herpotrichoides* with ampulliform conidiogenous cells giving rise to an aggregated, slimy mass of conidia.
Fig.2. Typical conidia of *P. aestiva* on SNA.
Fig.3. Conidium of *R. herpotrichoides* var. *herpotrichoides* attached to a polyblastic conidiogenous cell.
Fig.4. Older, swollen conidium of *P. aestiva* giving rise to a secondary conidium (arrow) via a conspicuous conidiogenous locus (double arrow).
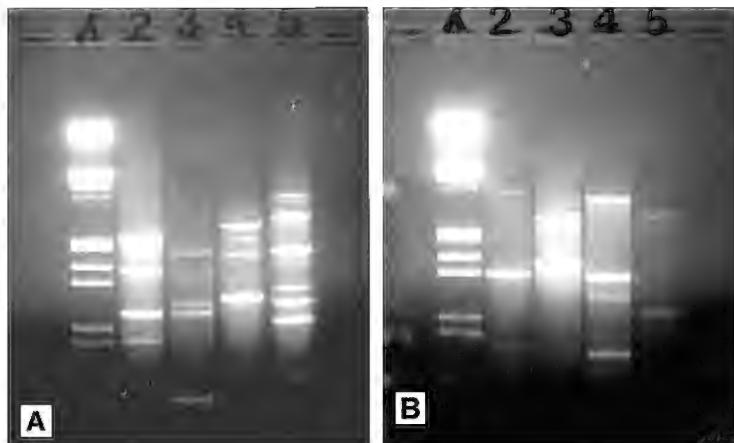
Fig.5. Polymorphic bands of amplified DNA of the three varieties of *R. herpotrichoides* and *P. aestiva*. Lane 1: Phage lamda DNA digested with EcoRI (A) and HindIII (B) was used as a standard. Lane 2: *R. herpotrichoides* var. *herpotrichoides*; Lane 3: *R. herpotrichoides* var. *acuformis*; Lane 3: *R. herpotrichoides* var. *anguioides*; Lane 4: *P. aestiva*.

	var. *herpotrichoides*	var. *acuformis*	var. *anguioides*	*P. aestiva*
var. *herpotrichoides*	X			
var. *acuformis*	50	X		
var. *anguioides*	17	9	X	
P. aestiva	9	29	8	X

Table 1. Combined F-values (% similarity) following RAPD- analysis (2 primers) of the three varieties of *R. herpotrichoides* and *P. aestiva*.

Using RAPDs, the present study supported these results, and also showed *P. aestiva* to be distinct from the three varieties of *R. herpotrichoides*. F-values between *P.aestiva* and var. *herpotrichoides*, var. *acuformis* and var. *anguioides* were found to be 9%, 29% and 8% respectively, indicating little relationship (Fig. 4, Table 1).

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In conclusion, the use of molecular techniques in *Ramulispora* is shown to enhance the taxonomic importance of seemingly insignificant morphological differences. Results obtained with SEM and RAPDs indicate that the four taxa studied are distinct, and that *P. aestiva* is probably not a species of *Ramulispora*. Furthermore, the low % percentage similarity obtained with RAPDs, as well as other morphological differences suggest that the three varieties of *R. herpotrichoides* should be treated as three separate species.

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DIVERSITY OF *COLLETOTRICHUM GLOEOSPORIOIDES* PATHOGENIC TO *STYLOSANTHES* SP. IN TROPICAL AREAS

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Abstract. Virulence spectra of *Colletotrichum gloeosporioides* strains isolated from *Stylosanthes* sp. sampled in Africa and Australia were investigated on a set of *Stylosanthes* genotypes. Virulence spectra as well as aggressiveness of strains were highly variable. The distinction of strains causing type A (restricted lesions) and type B (extensive necrosis) is confirmed. Esterases extracted from both types of strain showed isozymic variation. Polymorphism was observed between and within types ; the latter can be clearly separated by specific bands. Strains isolated from *Stylosanthes* sp. presented esterase patterns different from those of strains isolated from other tropical plants. By Random Amplified Polymorphic DNA (RAPD) technique markers have been identified which separate strains of types A and B. Polymorphism was also observed among strains of type B from various geographical origins.

Introduction

Stylosanthes guianensis is a tropical pasture legume very interesting for its tolerance to dry conditions in tropical areas. Since 1970's, anthracnose caused by *Colletotrichum gloeosporioides* Penz. is an important limiting factor in South America (Grof *et al.* 1979), Africa (Clatworthy 1975) and Australia (O'Brien and Pont 1977). Selection of resistant genotypes is commonly used to control anthracnose, but strains able to partially or totally overcome the resistance appear sometimes rapidly.

In order to understand the mechanisms of this pathogen-host coevolution and to assist in the collection and selection of resistant *Stylosanthes* genotypes, the diversity in pathogenicity, DNA and isozymic patterns of *C. gloeosporioides* strains from various hosts and origins is investigated.

Material and methods

Pathogenicity test

Virulence spectra of *C. gloeosporioides* strains isolated from *Stylosanthes* sampled in Africa and Australia (Table 1) were investigated on a set of *S. guianensis* genotypes by inoculation of conidia suspensions (10^5-10^6 conidia/ml) on first-leaf stage plants. Plants were kept 48 h in the dark at 23° C before further incubation under a photoperiod of 14 h. Symptom severity was quoted after eleven days.

Esterase assay

Esterases were extracted from mycelium and conidia grown in V8 liquid cultures in a Tris buffer at pH 7.5, loaded on non-denaturing gel for electrophoresis and stained with the a-naphtyl acetate/fast red TR salt method (Shaw and Prasad 1970).

DNA study

DNA was extracted by the CTAB method (Roger and Bendich 1985). The Random Amplified Polymorphic DNA (RAPD) was developed to distinguish strains, using primers from the Kit A of Operon Technologies Inc. and the DynaZyme polymerase (Techgen International).

Table 1. Host, type, geographical origin of *Colletotrichum gloeosporioides* strains

Strain	Host	Type[1]	Location
HM 334	*Stylosanthes sp.*	A	Australia
HM 335	*Stylosanthes sp.*	A	Australia
HM 336	*S. guianensis* cv. Graham	B	Australia
HM 337	*S. guianensis*	B	Australia
HM 313	*S. guianensis*	B	Burundi
HM 312	*S. guianensis*	B	Zaire
HM 373	*S. guianensis* cv Cook	B	Zaire
HM 376	*S. guianensis*	B	Zaire
HM 502	*S. guianensis* cv. Cook	B	Zaire
HM 495	*S. guianensis* cv. CIAT 184	B	Ivory Coast
HM 498	*S. guianensis* cv. CIAT 11366	B	Ivory Coast
HM 514	*S. guianensis* cv. CIAT 184	B	Ivory Coast
HM 515	*S. guianensis* cv. Cook	B	Ivory Coast

[1] Type of symptom from which the strain was isolated : A, restricted clear spots with a brown margin ; B, extensive dark brown necrosis.

Preliminary results and discussion

Pathogenicity

Two types of anthracnose, previously described by Irwin and Cameron (1978), are distinguished on diseased samples and in inoculation tests. Symptoms caused on the three genotypes inoculated with the strain of type A were characterized by a clear spot surrounded by a dark margin. Susceptible genotypes infected with strains of type B presented a general necrosis of plants, starting by dark brown spots extending on the leaflets, causing their premature shedding as also on the stem. Virulence spectra as well as aggressiveness of strains appeared highly variable (Fig. 1), symptoms induced ranging from a few small spots to death of plants.

Evaluation of the degree of necrosis from 1 (spots less than 1 mm in diameter) to 5 (more than 75 % necrosis of the leaf surface), is made by visual inspection of the first leaf inoculated. For each strain the results are expressed by the mean indice of the three leaflets of eighteen plants per genotype. A mean index is calculated for each genotype.

Esterases

Isozymic variation was observed for esterases extracted from both types of strain. Types could be clearly separated by specific bands. Polymorphism was also observed among strains of type B. Strains isolated from various other tropical plants

presented esterases patterns different from the strains isolated from *Stylosanthes* sp. Moreover, subcultures from sector appearing on PDA cultures of a *C. gloeosporioides* strain isolated from banana, showed different isozymic patterns.

DNA polymorphism
RAPD permitted to identify markers which clearly separate type A and B. Polymorphism was also observed among type B strains which could be grouped according to geographical origin
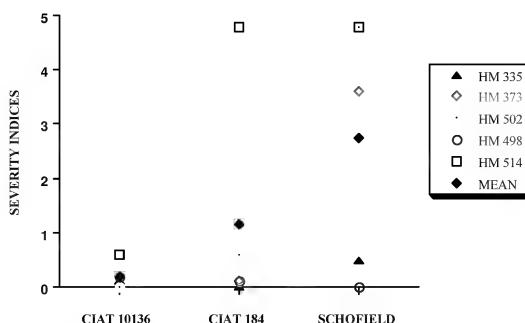
Figure 1. Anthracnose severity on the first leaf of three *Stylosanthes guianensis* genotypes, 11 days after inoculation with five *Colletotrichum gloeosporioides* strains

Strains from Australia, Burundi and Zaire were closely related while those isolated in Ivory Coast formed a second distinct group. Subcultures of strain HM 498 and from sectors of the HM 515 presented DNA polymorphism.

Prospects
Study of coevolution of *Colletotrichum gloeosporioides* and *Stylosanthes* sp. is planned in collaboration with the Universidad Nacional Autonoma Mexico and the Laboratorium voor Gentechnologie (Katholieke Universiteit Leuven) in the frame of a project coordinated by the International Board for Plant Genetic Resources (IBPGR).

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DIVERSITY IN NON-SPECIFIC FOLIAR PATHOGENS OF WHEAT FROM NON-TRADITIONAL WARM AREAS

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Abstract. In order to characterize the variability of the non-specific foliar pathogens of wheat from warm areas, a world-wide collection of strains is established through a collaboration between CIMMYT, Mexico and UCL, Belgium. By isolation of leaf samples sent from non-traditional warm area (South Asia, Africa, Latin America) in 1993 and 1994, we confirmed that *Bipolaris sorokiniana* is the most important foliar pathogen followed by *Drechslera tritici-repentis*, representing respectively 75 and 17% of the fungi isolated from leaf spots in 1993. This collection is the base for characterisation of the NSFP diversity by pathogenicity tests on host differentials of wheat lines developed at CIMMYT, by an analysis of the spectrum of toxins produced and by studies of genetic diversity with RAPD.

Introduction

Changes in cropping systems and progresses obtained in genetic resistance to classical diseases like rust, led to the important development of the non-specific foliar pathogens (NSFP) of wheat. With the support of the Belgian Agency of Development Cooperation, CIMMYT, Mexico and UCL, Louvain-la-Neuve collaborate to evaluate in warmer areas the sustainability of wheat production related to cropping systems and to promote research on resistance to this pathosystem.

In non-traditional warm wheat growing areas, helminthosporium blights, spot blotch and tan spot caused respectively by *Bipolaris sorokiniana* (Sacc. *in* Sorok) Shoem., teleomorph *Cochliobolus sativus* (Ito & Kurib.) Drechs. *ex* Dastur and *Drechslera tritici-repentis* (Died.) Shoem., teleomorph *Pyrenophora tritici-repentis* (Died.) Drechs., are considered as the most important foliar pathogens, the incidence of other pathogens such as *Alternaria triticina* being poorly documented (various authors in Saunders 1990).

Data concerning variability among strains are useful to assist selection of resistant or tolerant cultivars. New lines being selected in "hot spot" fields where the pathogen is naturally present at a high pressure, it is important to determine if variability of pathogenicity among the strains occurring in these fields is representative of variability found in other locations. Differences in virulence among strains of *B. sorokiniana* of a same country were shown i.e. for Thailand by Hetzler (1992) and for Brazil by Mehta (1981). Concerning *D. tritici-repentis*, different pathotypes producing different type of lesions in correlation with toxin production were described by Lamari and Bernier (1989).

A reference collection of pathogens from diverse origins is thus the first priority. This will be achieved through a collaborative network gathering UCL, CIMMYT and National Agricultural Research Systems from the warm wheat growing area.

Material and methods

Foliar samples were collected during 1993 and 1994 principally in the South Asian region (Bangladesh, Nepal and India) but also in South-Africa and Latin America in farmer or trial fields in characteristic locations on a great range of wheat varieties, some classical in these environments like Sonalika and Kanchan, some new resistant lines like crosses with *Agropyrum curvifolium*.
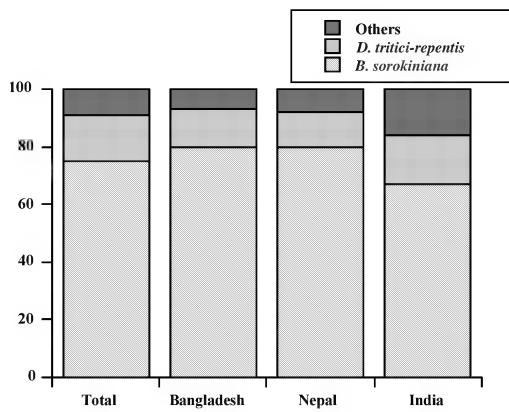
Figure 1: Distribution of the NSFP isolated from wheat in South Asian area in 1993: the number of strains isolated was 77 for all warm areas, 30 for Bangladesh, 26 for Nepal and 6 for India.

Lesions on wheat leaves and stems are observed under stereomicroscope and microscope to detect fungal fructifications.

After description, pieces of lesions are surface sterilized with NaOCl (0.5% during 3 minutes), rinsed with three changes of sterile water and placed in Petri dishes containing water agar (1.5%, streptomycin 150mg/l). After incubation in the dark at 20°C, the plates are placed under continuous light (15cm below a white and a near-ultra-violet tube) at room temperature to develop conidiophores and then in the dark at 20°C to induce sporulation. Conidia are transferred to fresh water agar plates until they germinate and then on V8-PDA plates. Monoconidial cultures are conserved in V8 slants under oil.

Results

Symptoms of foliar blights range from little black points to complete chlorosis and necrosis of the leaves, besides the characteristic symptoms of helminthosporium leaf blights: oval to elongated, light brown to dark brown blotches with or without a central dark spot.

The association of *Bipolaris sorokiniana* with the symptoms can be easily identified by the help of a stereomicroscope by the presence of dark conidia and for

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Drechslera tritici-repentis under a light microscope by the presence of conidia showing the characteristic conical basal cell (Drechsler 1923). However if no conidia are visible, seen the diversity of pathotype-genotype combinations and of environmental conditions, a distinction between leaf symptoms of the species is almost impossible without isolation.

In 1993, a majority of *Bipolaris sorokiniana* (75%) has been isolated from samples with foliar blights from warm areas. The rest is made up of 17% of *Drechslera tritici-repentis* and 8% of other fungi like *Curvularia* sp. and *Alternaria* sp (fig. 1). This distribution is globally the same in the particular environments analysed (Bangladesh, Nepal and India) and confirmed by the isolation of 1994.

Conclusion and prospects

This collection is the base for characterisation of the diversity of the non-specific foliar pathogens of wheat in warm areas.

Four items will particularly interest us for this characterization:
- time course evolution of the NSFP distribution in warm area by the annual visit and collect of samples in particular known locations and various cropping systems.
- variability in pathogenicity of the isolates from different location and year through inoculation test on host differentials of wheat lines developed at CIMMYT.
- variability in the spectrum of toxins produced by the isolates: non-host-specific *Bipolaris* toxins such as prehelminthosporal and prehelminthosporol (De Mayo *et al.* 1965); host-specific ones such as *Drechslera* toxins: necrosis and chlorosis inducing toxins (Ballance *et al.* 1989, Brown and Hunger 1993)
- genetic diversity with RAPD.

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PATHOGENIC DIVERSITY IN *MAGNAPORTHE GRISEA*, THE RICE BLAST PATHOGEN

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Abstract. Rice cultivation has been introduced in high elevation swamps of Burundi in 1980. Six years after, the released cultivar, Yunnan3, was severely attacked by *Magnaporthe grisea*, the rice blast pathogen. In order to identify resistant genotypes, race diversity encountered in this new rice growing area was studied. The results discussed in this paper have been obtained by inoculating 25 isolates of *M. grisea* on 27 rice cultivars including 13 Japanese differentials. All the known resistance genes are broken down by Burundian isolates except Pi-ta^2. A high diversity is thus established.

Introduction

Blast, caused by *Magnaporthe grisea* (Hebert) Barr (anamorph *Pyricularia oryzae* Cavara), is one the most widespread disease of rice in tropics. All the rice breeding programmes are aimed at controlling that disease by resistant cultivars.

As a preliminary to resistance breeding for blast, the pathogen variability has to be well known. Virulence spectrum of isolates collected from rice growing areas has to be determined.

In developping countries where rice is included in the subsistence crop system-a low input one- vertical resistance, which is race specific, would be avoided since the pathogen adapts to such cultivars very quickly. Moreover, when severe epidemics break out, the farmers don't have means to afford fungicides. Hence, more efforts must be done to select quantitative resistance which ensures durable protection and more stable yield.

In Burundi, rice has been introduced in high elevations in 1980 with the release of Yunnan 3. Six years after, in 1986, high damages were caused to this cultivar by neck blast. Therefore, blast is taken into account in resistance breeding.

A collection of isolates was made up and a high diversity was observed by inoculation on differentials and some reference cultivars from various rice growing areas. Results of those tests are discussed below.

Symptom description

Magnaporthe grisea infects rice at all growth stages, resulting in two types of symptoms: leaf blast and panicle blast.

The leaf spots are typically elliptical with usually grey or whitish center and a brown or reddish-brown margin. The shape and colour vary depending upon the degree of susceptibility of the cultivars and the environmental conditions. Highly resistant cultivars develop minute brown specks of pin head size, while susceptible ones growing in conducive conditions show no or very little brown margin.

Panicle blast can affect any part of the panicle but often its base, causing the rotten neck also called neck rot. Panicles often fall over and grains are empty. Panicle branches and glumes can also be attacked. This type of blast is the most detrimental to yield.

Material and methods

Twenty five isolates of *M. grisea* including 20 from Burundi (BD) and 5 from Cameroon (CM28), Columbia (CL6), Ivory Coast (CD45), Korea (CR5) and Philippines (PH11) were grown on rice flour agar medium (1 litre of water, 15g of agar, 20g of rice flour) in Petri dishes at 28°C; 60% of relative moisture and 12 h of light per day. After 10 days of fungal growth, a spore suspension adjusted to 25,000 conidia/ml was injected between leaf sheaths with a hypodermic syringe.

Thirteen differentials carrying different resistance genes and 13 reference cultivars were used in this experiment. Plants were grown in a greenhouse up to 4-5-leaf stage in batches containing compost and watered with nutritive solution.

Seven days after inoculation, lesions were assessed using Notteghem's 6-class scale where scores 1-3 correspond to resistant cultivars, 4-6 to susceptible ones.

Results

All the resistance genes carried by differentials are broken by Burundian isolates except Pi-ta^2 of Pi n°4 (Table1). Isolates CL6, CM28 and CR5 from respectively Columbia, Cameroon and Korea overcome Pi-ta2 gene. PH11, CL6, CM28, CR5 and CD45 are highly aggressive on IR841, IR8, L9 and Mafushi which are resistant to Burundian isolates (Table2).

BD29, BD30, BD32, BD37 and BD38 attack Tetep which is widely used as resistant donor in most of the rice breeding programmes. On IRAT13 only moderate type lesions are observed. All the isolates severely infect Yunnan 3.

Discussion and Conclusion

There is a high pathogenic diversity in the *M. grisea* population from high elevation swamps in Burundi despite the limited number of rice genotypes grown in this environment. All the known resistance genes are broken down by Burundian isolates except Pi-ta^2. The latter is, however, matched by isolates from other geographic areas. In addition, all the isolates have overcome five genes or more. Improving resistance strategies based on these genes separately or in combination can thus not be effective for a long time. This suggest that in Burundian context, cultivars carrying only major genes should be avoided even those carrying Pi-ta^2 since material exchange is not rigorously controlled.

More emphasize should be put on quantitative resistance which is often durable. Cultivars with a good level of quantitative resistance such as IRAT13, L9 and Mafushi should be used as parents. Promising progenies should be tested in a large number of rice growing countries and tests would be repeated during several seasons.

Recent molecular techniques especially Restriction Fragment Length Polymorphism (RFLP), have enabled to map blast resistance genes conferring quantitative resistance (Wang et *al.* 1993). Using that tool should help to find out quantitatively resistant cultivars to broaden the resistance background.

Other workers (Notteghem 1993, Wang *et al.* 1993) propose combining a quantitative resistance with a qualitative one to face *M. grisea* variability.

To carry out this breeding, it is essential to understand the *M. grisea* population structure. Appearance of new isolates and their spectrum should be monitored. DNA markers identification has already proved its efficiency (Wolfe 1993).

Table 1: Response of 13 rice differentials to inoculation with 25 isolates of *M. grisea*

CULTIVAR	Isolates from various origins					Burundian isolates																			
	PH 11	CL 6	CM 28	CR 5	CD 49	BD 13	BD 16	BD 18	BD 24	BD 25	BD 26	BD 27	BD 28	BD 29	BD 30	BD 31	BD 32	BD 33	BD 34	BD 35	BD 36	BD 37	BD 38	BD 39	BD 40
SHIN 2	-	6	6	-	4	-	5	6	6	5	6	5-6	6	6	6	5	6	5	3-4	5	6	6	5-6	6	6
AICHI ASAHI	5	6	6	6	5	6	5	6	6	5	6	6	6	5-6	6	5-6	4	6	5-6	6	6	6	5	6	6
FUISAKA n°5	6	6	6	5	5	6	5	6	6	6	6	5-6	6	6	6	-	6	3	1	6	6	6	6	6	6
KUSABUE	3	6	5	3	4	6	5	3	6	6	3	6	6	6	6	6	4	4-5	6	4	6	5-6	4-5	5-6	6
TSUYAKE	1	6	6	6	1	4	6	2	5	6	4	6	5	6	5	3	6	6	6	6	5	5	6	6	5
FUKUMISIKI	4-5	5	3-4	6	4	3-4	6	3	1	3-4	4	4	4-5	1	5-6	4	6	6	3	3	2	4	4	6	1
K 1	1	5	1	6	4	1	6	3	6	3	1	6	6	6	6	3	1	6	6	6	1	2	6	6	5
P t n°4	3	6	6	6	1-2	3	1	2	1	3	1	1	1	1	1	1	6	1	2	1	1	1	-	6	3
TORIDE n°1	5	1	6	1	2	1	1	1	6	1	1	6	6	6	6	6	1	2	2	3	6	5	6	6	5
K 2	6	6	6	6	1	6	6	6	6	1	2	1	2	6	6	1	6	3-4	2	3	6	5	3	6	6
BL1	2	5	5	4	1	3	2	3	3	3	3	6	6	3-4	6	6	1	4	6	3-4	2-3	3	3	3	3-4
K 59	1	1	1	5-6	1	5	1	3	3	3	3	4	5-6	3	2-3	3-4	6	3-4	4-5	5	3	1	3-4	1	1
K 3	1	3	6	1	2	4	1	3	1	1	2	3	3	4	1	3	4	1	4	5	1	3	4	2-3	3
MARATELLI	6	6	6	6	6	6	6	6	6	6	1	6	3	6	6	6	6	6	6	6	6	6	6	6	6

Table 2: Response of 13 reference cultivars to inoculation with 25 isolates of *M. grisea*

CULTIVAR	Isolates of diverse origins					Burundian isolates																			
	PH 11	CL 6	CM 28	CR 5	CD 49	BD 13	BD 16	BD 18	BD 24	BD 25	BD 26	BD 27	BD 28	BD 29	BD 30	BD 31	BD 32	BD 33	BD 34	BD 35	BD 36	BD 37	BD 38	BD 39	BD 40
YUNNAN 3	5	6	6	6	5-6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	-	6	6	6	6
IRAT 13	3	2	3	3	2	1	1	1-2	3-4	1	1	3	2	3	2	2	2	1	1	4	2	2	4	3	2-3
IR 841	6	6	6	1	-	1	5	4	1	1	3	2-3	1	3	1	1	1	1	1	3	1	1	6	1	6
TELORIRANA	5	4-5	4-5	4	6	3	1	3-4	6	1	1	6	3	6	1	6	6	3	3	3	3-4	4	6	6	4
IR 8	6	6	6	6	6	1	5	2	1	3	1	2	2	1	1	1	1	1	1	3	1	1	4	6	4
IR 5	4	6	6	6	6	4	4-5	4	6	4	4	6	6	5	5	6	6	3	1	1	4	3-4	5-6	6	5-6
CARREON	1	1	4-5	1	1	1	4	1-2	1	1-2	1	1-2	1-2	1	1	1	1	3	2	1	4	1	2	1	3
TETEP	1	3-4	6	6	6	5	3	2	4	2	2	3	3	6	5-6	5	6	3	2	3	3	4-5	4	5-6	7
KIRUNDO 3	6	5	6	2	1	1	3	3	6	1	2	6	5	2-3	3	5	5	3	3	4-5	3	3-4	5	4	3
L 9	6	1	6	1	4-5	5	2	2	3	1	4	4	3	2-3	4	3	3	1	1-2	3	1	1-2	2	2	1
NP 125	5	6	6	4	3-4	3-4	1	3	5-6	1	1	4	4	2-3	6	4	6	1	6	6	3-4	3	4	3-4	3-4
CHIANAN 8	3	3-4	3-5	1	-	5-6	5	4	6	1	1	5	6	6	3	6	6	1	6	2	4-5	1	6	5	1-2
MAFUSHI	-	6	6	1	-	6	1	1	6	1	1	1	2	2	2	2	2	6	1	1-2	-	1	6	1	5
MARATELLI	6	6	6	6	6	6	6	6	6	5-6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

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CHARACTERIZATION OF *POLYMYXA GRAMINIS* INVOLVED IN THE TRANSMISSION OF PEANUT CLUMP VIRUS IN TROPICAL AREAS

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Abstract. The plasmodiophoraceous fungus, *Polymyxa graminis* Ledingham, is involved in the transmission of Peanut Clump Virus (PCV), a soil-borne virus widely distributed in India and in West Africa. This fungus is known in temperate areas to be the vector of soil-borne viruses on barley, wheat and oat. Ecological requirements of *P. graminis* isolates from various origins are possibly different. Indian and African isolates indeed grow easily at 25-30°C, whereas those from temperate areas are favoured by temperatures between 15 and 20°C. Host range of tropical isolates is apparently wider and less specific than that of isolates from temperate areas. Indian *P. graminis* isolates grow on monocotyledonous plants as well as on dicotyledonous plants, whereas in temperate areas, two fungal species are listed as separate species according to the host specificity; *P. graminis* growing only on graminaceous plants and *Polymyxa betae* Keskin on Chenopodiaceae. In order to clarify the taxonomic position and precise the requirements of *P. graminis* associated with PCV transmission, pure strains obtained from a single cystosorus are prepared from isolates of various origins. Host range, ecological requirements, such as temperature optimum and pH, are studied. First results confirm differences in the temperature optimum, the Indian strain growing best on sorghum between 20 and 30°C compared to less than 20°C for the strains from northern origins on barley.

Introduction

Peanut clump is a soil-borne virus disease widely distributed in India and West Africa. Infected plants are stunted conspicuously and have dark green leaflets. Flowers can be produced, but any pods formed are poorly developed (Thouvenel *et al.* 1976; Reddy *et al.* 1983). The virus is transmitted with seed from groundnut, pearl millet and finger millet infected plants, but is also soil-transmitted by a plasmodiophoraceous fungus, *Polymyxa graminis* Ledingham, an ubiquitous obligate root parasite of graminaceous plants (Thouvenel *et al.*, 1976; Thouvenel & Fauquet 1981, Ratna *et al.* 1991). The resting spores of the fungus may contain the virus and can survive in soils for many years. In specific conditions, spores

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germinate and zoospores infect epidermic root cells of a host plant. The plasmodium, the zoosporangium, producing secondary zoospores, and the cystosorus, the survival stage of the fungus, are all formed in the infected cell.

In temperate areas, two fungal species are distinguished according to the host specificity : *P. graminis* growing only on Poaceae (Ledingham 1939, Barr 1979, Bastin *et al.* 1989, Adams 1990), and *Polymyxa betae* Keskin on Chenopodiaceae and some related families (Barr 1979, Barr and Asher 1992). The two species are known to be the vector of soil-borne viruses on their specific hosts. In India, *P. graminis* isolates grow on monocotyledonous plants, such as pearl millet, sorghum and wheat, as well as on different families of dicotyledonous plants including Chenopodiaceae. According to this wider and less specific host range, the taxonomic position of the *P. graminis* strains associated with peanut clump virus transmission is questionable. Is it the same species as *P. graminis* found in temperate areas or is it a third one of the same genus, able to infect and transmit virus(es) over a wider host range? Is the host specificity a relevant criterion to separate species?

The in depth understanding of the *P. graminis* populations involved in the transmission of the peanut clump virus and the acquisition of basic information for development of an integrated control of PCV at small holdings level, are the main objectives of the collaborative research project between Université catholique de Louvain - Unité de Phytopathologie and International Crops Research Institute for the Semi-Arid Tropics.

The program started in March 1993 with the isolation of *P. graminis* from various origins and the production of single cystosorus strains. The characterization of the *P. graminis* populations involved in the transmission of the peanut clump virus and the comparison with strains from temperate areas are in progress. The results presented here concern a first experiment on the temperature requirements for 5 strains from various origins.

Materials and methods
Single cystosorus cultures

P. graminis isolates were obtained by growing bait plants in infested soils from various origins. Pieces of rootlets containing *P. graminis* cystosori were soaked in sterile distilled water, cut in 1 or 2 mm fragments and homogenized in Omnimixer Virtis at 25000 rpm. Few drops were spread on 2% water agar in Petri dishes. The surface was carefully analysed with a Wild Macroscope at 40-100 x magnification to detect single cystosorus.S ingle cystosori were taken with a micro spear and placed adjacent to the roots of a 2-day-old pregerminated seedling planted in single tube on sterile quartz sand. Hundred to 300 plants were each inoculated with a single cystosorus for each isolates. The plants were watered with half-strength nutrient solution and grown at 15-20°C (night-day) or 25-30°C according to the temperate or tropical origin of the *P. graminis* isolates. After 2 or 3 months, each plant was removed and checked under the microscope to detect the fungus. Infected roots were dried and stored at room temperature.

Fungal multiplication was performed in an automatic immersion device. The latter was constituted by PVC tubes suspended in a container, linked with a pump to an independent nutrient solution tank. Host plants were planted in the tubes containing sterile quartz sand inoculated with root extract infested with the *P. graminis* strain. The basis of each tube was formed by a polyamid grid allowing flowing in and out of nutrient solution and zoospores. Watering is programmed

during 6 hours each 12 hours. After 2 months, plants were removed and the roots observed to ascertain the presence of the fungus. The strains are stored in dried root fragments at room temperature.

Temperature requirement

Five single cystosorus strains were tested : strains I1-20 and I1-229 from India multiplied in sorghum roots, and the strains B1 from Belgium, C1 from Canada and F11 from France in barley roots.

Culture tubes filled with sterile quartz sand were inoculated with cystosori suspensions of each strain (1000 cystosori/tube). The effect of temperature on the development of temperate and tropical strains, respectively on barley or sorghum, was tested by transplanting 2-day-old seedlings in inoculated sand and growing them at the test temperatures, 10-15°C, 15-20°C, 20-25°C, 25-30°C or 30-35°C (night/12h-day/12h). Periodically, 4 plants grown at the various temperatures were removed for each strain. The roots were coloured in blue lactophenol and observed under the microscope for assessment of fungal infection.

First results

Up to now, six *P. graminis* strains have been obtained and multiplied: three Indian strains on sorghum and isolated from a soil sampled at Patancheru in a field infected by the peanut clump (strain I1-1, I1-20, I1-229), one Belgian strain on barley and isolated from a soil sampled at Loupoigne in a field infected by the barley yellow mosaic (strain B1), one Canadian strain on barley obtained from barley roots grown on Ottawa soil in 1987 and send by Dr D. J. S. Barr (strain C1), and one French strain on barley and isolated from a soil sampled in 1988 at Carcassonne (strain F11).

Temperature requirements for *P. graminis* infection are different according to the origin of the strains (Fig. 1). The two Indian strains have a narrow optimum temperature around 25°C. Below 20°C and above 30°C, any trace of infection was not yet observed after six weeks. Strains from temperate areas are favoured by cooler temperature: the strains B1 and C1 grew easily between 10 and 20°C, and the strain from the south of France grew only at 15-20°C, 5 weeks after the inoculation.

Differences in infection capacity are also evident. Belgian strain B1 showed 100% infection on barley after 2 weeks, whereas minimum 4 weeks of incubation are necessary to the strain C1 to reach this level. The 3 other strains seem to have a lower infection capacity, especially the strains coming from India.

Discussion and prospects

The first results demonstrate differences in the temperature requirements of *P. graminis* strains according to the origin, the Indian strains being able to develop at temperatures inhibitory to the Belgian strain. The results with the Canadian and the French strains suggest a continuous range of temperature optimum. Further repetitions of these experiments will specify the differences in response at the various growth stages of the fungus and the influence of the host in the observed differences. In view to a better understanding of the *P. graminis* populations involved in the transmission of the peanut clump virus, soil water potential and pH requirements will also be tested. The host ranges including dicotyledonous plants and weeds of the tropical strains will be analysed and compared to temperate *P. betae* and *P. graminis* species. A morphological analyse of the various stages of the

fungus and a genomic analyse of the various strains on DNA fragments am plified by PCR will be done to define the taxonomic position of the fungus involved in the PCV transmission.

Acknowledgements
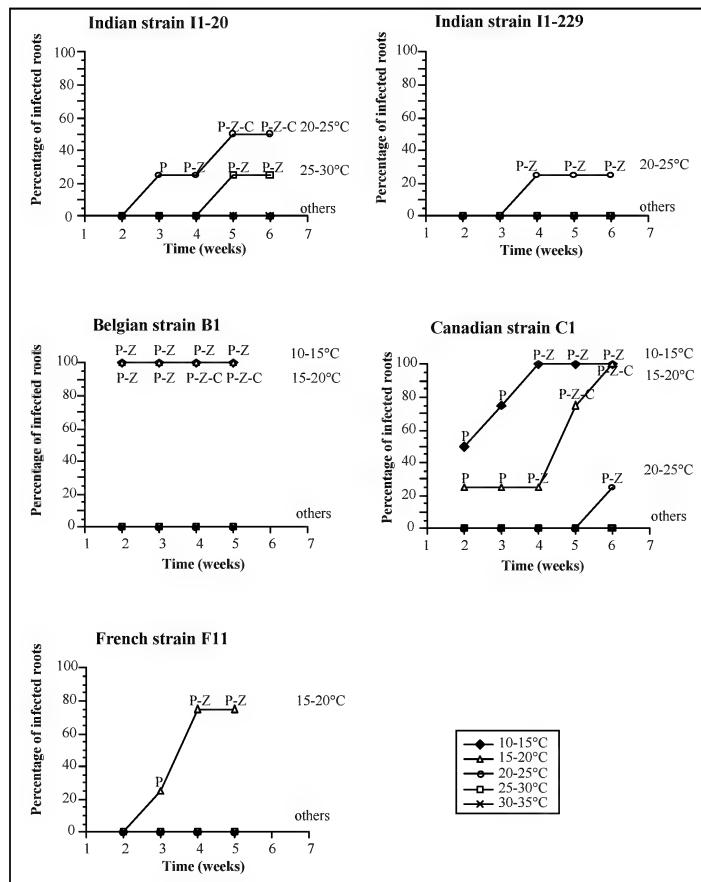
Fig. 1 Comparison of temperature requirement of Indian *P. graminis* strains (strains I1-20 and I1-229 on sorghum) and temperate strains (strains B1, C1 and F11 on barley). Fungal stages observed in the roots: P = plasmodium, Z = zoosporangium and C = cystosorus.

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PRACTICAL MYCOLOGY

PRACTICAL ADVICE FOR COLLECTING WOOD-ROTTING FUNGI IN THE TROPICS

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Number of new species of mainly wood-rotting fungi of Aphyllophorales is increasing rapidly. Nevertheless, only a small part of the tropical territories has been mycologically explored; thousands of species have not yet found and described. Type specimens and other collections of Aphyllophorales collected earlier are in many if not most cases sterile (without basidia and spores). Their original consistency and colour have usually changed, fruit-bodies are sometimes badly damaged by insects. Comparatively very small number of species has been introduced to pure culture.

For developing modern taxonomy of this economically important group of fungi, extensive collecting of specimens is needed. Some practical advises in addition to the ones published in several handbooks are given below.

1. When sampling a specimen growing on wood, the tree species (or at least genus) must be identified if possible: there are externally similar sibling species specialized in several genera of wood-rotting fungi *(Phellinus, Inonotus, Peniophora, Corticium* etc.). Sample also a piece of rotten wood (substrate) or describe the rot type (white, brown, pocket rot...).

2. Describe the colour of all parts of the fresh basidiomata (fruit-bodies) as soon as possible using standard colour charts (e. g., the rather cheap "Methuen Handbook of Colour" by A. Kornerup & J.H. Wanscher). Note colour change after bruising (handling) the basidioma.

3. Dry the specimens in the same day you collected them. When kept moist or dried slowly, basidia will collapse and spores will be damaged or destroyed (by bacteria?).

4. Separate a part of the big basidioma (or one of the basidiomata) to take a spore print. This is extremely important! If the collected basidioma is dry, put it (in the evening) for 10-30 min. into water. After some drying (basidioma must be moist but not wet) put it on a piece of black and white paper collection number written on it. Hymenophore (pores, lamellae, folds) must be facing downwards. Put the basidioma with the paper strip below it for the night in a plastic bag to avoid desiccation. Next morning you can usually get a good spore print; in several cases, sterile when collected specimens have developed new basidia and began to sporulate. Dry the basidioma used for taking a spore print, and mark it: it may be the best part of your collection. The piece of paper with spore print must be dried promptly (with no heating) and kept in a labelled envelope.

5. Spore prints when kept dry (if possible, in frigerator at 2–5° C) may be used even after one or some weeks to inoculate suitable medium in your lab to get a pure culture. In some species, spores are viable up to several months.

6. When studying herbarium specimens microscopically, use the spore print to measure 25-50 randomly taken spores to calculate mean spore size and spore length/width index Q.

7. After returning from a collecting trip, disinfect your collections in a deep-freezer. Keep them at -18° C (better: at -25° or even -30°) 1-2 days, in big fruit bodies of polypores - up to 3 days. If you put every disinfected specimen in a minigrip plastic bag, further infection by insects is surely avoided. Use of poisonous disinfectants must be avoided: some of these are almost useless (as is the frequently used in tropical herbaria naphthalene), all are dangerous for health, and many of them may disturb the use of specimens in molecular taxonomy studies later.

8. If you have found an interesting collection you consider to belong to a new species, do not hurry describing it as a new one. About 70-75 % of "new" species are based on only *one* collection nowadays. In many cases these are not new *species* but "deviating" *specimens* of old ones of unknown variability. Keep in mind that variation of size of spores, basidia, cystidia, pores etc. in one specimen does not give any information on variability of the species. Avoid increasing taxonomic "noise," better continue collecting in other areas to find more specimens of your fungus.

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THE INFLUENCE OF PREPARATION ON ASCOSPORE
MEASUREMENTS

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Abstract. The influence of various media (tap water (TW), 2% potassium hydroxide solution (KOH), cotton blue solution in lactophenol (CB), polyvinyl lacto-phenol (PVLPh), detergent solution in water (DE), fixative (FAA)) and the storage on the ascospore measurements of *Byssonectria terrestris* (Alb. & Schw.:Fr.) Pfister, *Neotiella vivida* (Nyl.) Dennis & Rifai and *Peziza domiciliana* Cooke were compared. The influence of the medium and the storage on spore dimensions of different species has the same trend but its effect may be different. The comparison of spore dimensions taken from spore print, from rehydrated herbarized material and from living material immediately or up to 45 days after preparation is not justified.
Additional keywords. Ascomycetes, spore measurements, microscopy.

Introduction

In the systematics of Ascomycetes spore measurements as quantitative characters are used for the delimitation of populations and species, for the study of geographic speciation and for the construction of phylogenetic trees (Huhtinen 1989, Fogel 1992, Möls Raitviir 1974, Schumacher 1990, Wu 1993). The natural variability of spore dimensions of a specimen may be considerable, and recorded variability always includes measurement errors. These errors have two main causes: 1). errors of the measurement procedure itself, e.g. due to the orientation of a spore in a microscopic specimen (Rahi Kullman 1993); 2) spore shape distortions (shrinkage or swelling) due to differences in the osmotic concentration (water potential) of the spore content and the medium.

There is strong experimental evidence that spore dimensions depend on the type and concentration of the fixative, medium as well on spore/specimen storage. Spore shrinkage up to 26% in linear dimensions in the Melzer reagent (56% of the calculated volume) has been reported (Baral 1992); these data indicate that one should be extremely cautious in comparing spore measurement data when details of the measurement procedure are incomplete or missing. Dealing with data on type specimens this recommendation is essential but sometimes unrealistic.

Considering the huge amount of published data on spore dimensions it is of interest to evaluate effects on spore dimensions due to: 1) type and storage of a specimen (fresh, air dried herbarized fruitbody or spore print); 2) type of medium.

Material and methods

The influence of the following media on spore dimensions were evaluated: tap water (TW); 2% potassium hydroxide solution (KOH); 0.2% cotton blue solution in lactophenol (40%) (CB); polyvinyl lactophenol containing 56% of polyvinyl alcohol, 22% of lactic acid, 22% of phenol (by volume) (PVLPh); fixative water solution containing 4% of formalin, 23% of ethanol, 1% of acetic acid (FAA); detergent solution (some drops of Fotoflo 200 in 100ml 1% NH_3 solution - DE). The effect of the time of taking spore measurements (immediately or up to 45 days after preparation) and the type of a microscopic specimen (made from spore print, fresh, fixed or rehydrated herbarized fruitbody) on spore dimensions were also examined. The species under study were: *Byssonectria terrestris* (Alb. & Schw.:Fr.) Pfister

(spore print from several fruitbodies of one specimen, 8 fresh and 17 herbarized fruitbody), *Peziza domiciliana* Cooke (spore print from one fruitbody) and *Neotiella vivida* (Nyl.) Dennis & Rifai (fixed fruitbody).

From each fruitbody/spore print 25 spores were measured with an ocular micrometer (microscope "Amplival", immersion objective HI 100) with an accuracy of ±0,3 μm. A database of spore dimensions was compiled. The mean spore length (L) and width (W), L/W ratio (Q), standard deviation (s) and the coefficient of variation (V) were computed for each sample.

In the analysis of variance "Statgraph" procedures were applied: One- and Two-Way ANOVA for means and variances; Analysis of the Nested Design for evaluating the effects of the medium and the fruitbody. The significance level 0.05 was applied throughout the analyses.

Results

The spores of *Byssonectria terrestris* from one specimen measured in CB were examined. One-Way ANOVA revealed that a) samples from the same herbarized fruitbody do not differ significantly (p > 0.3) and b) spore samples from the same common spore print of several fruitbodies do not differ significantly (p > 0.9). Variance of spore means is the smallest in spore print (Table 1. Fig.1 A) which may serve as a "reference standard" in comparative study (Table 2).

The spore dimensions of a fixed *Neotiella vivida* fruitbody do not change significantly in TW, while they diminish essentially in CB.

Nested Design ANOVA revealed significant temporal differences spore dimensions measured on a fresh fruitbody and half a year later on the same dried specimen (FFinTW and HFinTW; FFinTW and HFinCB) and also significant difference spore dimensions measured in CB on a spore print and on the herbarized specimen (SPinCB and HFinCB).

Table 1. Spore measurements (L, W, Q) of spore print (SP), fresh (FF) and herbarized (HF) fruitbodies of one *B. terrestris* specimen; coefficients of variation (V_L, V_W) and standard deviations (s_L, s_W) of sample means (_, _); coefficients of variation of samples (V_l, V_w) in different media (TW, CB). Samples from the same common spore print of several fruitbodies - *; samples from the same fruitbody -°.; samples from the several fruitbodies - ▪.; N-number of samples (of size 25)

	N	L	V_L	s_L	W	V_W	s_W	V	V_W	Q
*SPinCB	4	18.8	0.4	0.1	8.7	0.3	0.03	4-5	3-5	2.17
°HFinCB	5	18.8	1	0.2	7.9	2	0.2	4-6	7-11	2.38
▪FFinTW	7	20.0	1	0.1	8.5	1	0.1	4-11	3-5	2.35
▪HFinCB	5	18.6	2	0.3	7.8	2	0.2	4-5	6-9	2.39
▪HFinTW	7	18.5	3	0.5	8.0	3	0.2	3-4	4-6	2.31

Tables 2-4. Mean spore measurements (L,W,Q) of spore samples (n=25) and their coefficients of variation (V), standard deviations (s) in different media; differences of these measurements with respect to those measured in CB (Tables 2, 3) or in FAA (Table 4) (%, μm) and the significance level (p) of the difference.

Table 2. *Byssonectria. terrestris* from the same common spore print of several fruitbodies.

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Media		µm	s	V	µm	%	p	Q
CB	L	18.8	0.8	4				
	W	8.7	0.4	4				2.17
PVLPh	L	18.9	0.7	4	+0.1	0	0.64	
	W	8.5	0.4	4	-0.2	2	0.06	2.22
FAA	L	18.7	0.6	3	-0.1	1	0.51	
	W	8.6	0.3	3	-0.2	1	0.32	2.18
KOH	L	19.0	1.0	6	+0.2	1	0.28	
	W	8.9	0.5	6	+0.2	2	0.03	2.14
TW	L	19.5	1.0	5	+0.6	3	0.00	
	W	9.1	0.4	4	+0.2	5	0.00	2.13
DE	L	19.5	1.1	5	+0.7	4	0.00	
	W	9.5	0.6	7	+0.8	9	0.00	2.10

Table 3. *Peziza domiciliana* from the same common spore print of one fruitbody.

Media		µm	s	V	µm	%	p	Q
CB	L	14.8	0.7	4				
	W	9.0	0.7	8				1.65
PVLPh	L	14.5	0.6	4	-0.3	2	0.07	
	W	8.2	0.4	5	-0.8	8	0.00	1.76
TW	L	14.9	0.6	4	+0.1	1	0.89	
	W	9.6	0.5	5	+0.6	6	0.00	1.56

Table 4. *Neotiella vivida* from the one fixed fruitbody

Media		µm	s	V	µm	%	p	Q
FAA	L	23.8	1.2	5				
	W	13.1	0.6	4	1.8	3		
TW	L	23.6	0.9	4	-0.2	1	0.43	
	W	13.4	0.7	5	+0.3	2	0.10	1.76
CB	L	21.8	0.9	4	-2.0	8	0.00	
	W	12.0	0.6	5	-1.1	8	0.00	1.82

Table 5. Analysis of Nested Designs. Variance components of the medium (ME%), fruitbody (FB%), and the residual (Spore%) for spore length (L) and width (W) on the basis of the *B. terrestris* spore measurements of fresh (FF) and herbarized (HF) fruitbodies and the spore print (SP) as compared in different media CB, TW, TW_CB. If $F_{emp} > F_{kr}$, the variance component is significant at a=0.05.

		ME%	F_{emp}		F_{kr}	FB%	F_{emp}		F_k	Spore%
FFinTW and HfinTW	L	53	63.0	>	4.8	4	3.5	>	1.8	43
	W	40	25.0	>	4.8	9	5.6	>	1.8	51
FFinTW and HfinCB	L	46	91.5	>	5.3	0	1.2	<	3.8	54
	W	43	55.7	>	5.3	2	1.8	<	3.8	5
HFinTW and HFin CB	L	2	1.3	<	5.3	21	7.9	>	3.8	77
	W	2	1.5	<	5.3	14	5.1	>	3.8	84
HFinTW and HfinTW-CB	L	5	1.7	<	5.3	34	15.0	>	3.8	61
	W	8	2.7	<	5.3	23	7.5	>	3.8	69
SPinCB and HfinCB	L	3	2.7	<	6.0	4	2.1	<	2.1	93
	W	67	456	>>	6.0	0	0.4	<	2.1	33

The temporal changes in spore dimensions of *Byssonectria terrestris* are illustrated

in Fig. 2. Changes of spore width measured in spore print in PVLPh are mixed: a small but significant decrease is followed by an increase. A significant decrease in spore dimensions was observed in a microscopic specimen of a fruitbody and a spore print mounted on PVLPh and CB respectively. Decrease is not significant up to the 6th day in spore print in CB.

Discussion and recommendations

1. Spores discharged from the ascus are significantly wider in comparison with spores in fruitbodies (Table 5: SpinCB, HFinCB. Fig.1: $L_4 > L_E$ or $L_A > L_B$).

2. Spore dimensions decrease with the storage of a herbarized specimen. Spores in fruitbodies measured a half year later are significantly smaller in comparison with spores in fresh fruitbodies (Table 5: FFinTW and HFinTW; FFinTW and HFinCB. Fig.1: E>C). Weekly drying does not cause any essential change (E and 6).

3. Preliminary rehydration of a herbarized specimen in TW increases significantly the variance of means of spore dimensions in comparison with those measured on spores introduced directly into CB (Table 1. Fig. 1: C,D - B). The means of spore dimensions of fresh fruitbodies (Table 1. Fig.1: E) vary less than those of herbarized fruitbodies.

4. Variance of the spore means is the smallest in spore print (Table 1. Fig.1: A) which may serve as a "reference standard" in a comparative study. The spore discharge as a pretreatment factor probably account for this homogeneity.

5. Considering effects on spore dimensions and variability (Table 2. Fig.1: A, 1-5) on the wall structure, the media investigated can be divided as follows:

a) FAA, CB, PVLPh -impact on spore sizes and variability is minor. Preference should be given to FAA;

b) TW - causes no wall disintegration, but impact on dimension variability is greater. This may be caused by different spore viability (Stojanovic, Ristanovic and Jencic 1986);

c) KOH, DE - due to disintegrating effects on the spore wall the impact on dimension variability is the greatest. The effects of KOH, TW and DE on spore dimensions are significantly greater than that of CB.

6. The effect of various media on the length/width ratio (Q) differs, Q is maximal in PVLPh and minimal in DE. Therefore, one must be extremely cautious when comparing data on Q (Table 2).

7. The essential increase in TW is the same for spore measurements of *Byssonectria terrestris* and *Peziza domiciliana*. Differences in its effect are probably due to different spore wall rigidity (Table 2 and 3).

8. The fixative seems to preserve the measurement of both dry spores and those taken from live fruitbodies. On the contrary in CB, it dehydrates spore in live fruitbodies (see *B. terrestris* spore print, Table 2. and a fixed *N. vivida* fruitbody, Table 4).

9. In microscopic specimens from fresh fruitbodies and spore print mounted in CB and PVLPh temporal changes in spore dimensions may differ considerably. In microscopic specimens of spores in PVLPh the distance between glass surfaces decreases due to the medium loss by evaporation and spores may become compressed and distorted. In microscopic specimens from fruitbody these effects do not take place.

It is recommended to measure spores in CB in the spore print and in the herbarized fruitbody during the first six days (Fig.2-4).
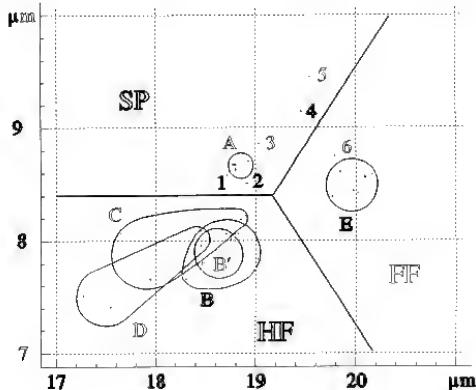
Fig.1. Mean spore length and width in one specimen of *B. terrestris*. SP - spore print, FF - fresh fruitbody, HF -herbarized fruitbody. Points: 1 - in fixative (FAA), 2 - in polyvinyl lacto-phenol (PVLPh), 3 - in potassium hydroxide solution (KOH), 4 - in tap water (TW), 5 - in detergent solution (DE), 6 - one week storage of the fruitbody in TW, clusters A - spore samples of the same common spore print from different fruitbodies in CB; B - samples from different fruitbodies in CB; B' - samples from the same fruitbodies in CB; C - rehydrated samples in TW, D - rehydrated samples mounted in CB; E -samples from the fresh fruitbody in TW.
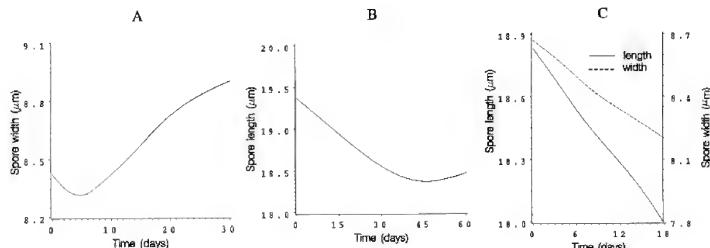
Fig. 2. Temporal changes in spore dimensions in a microscopic specimen of *B. terrestris*. Spore print mounted in polyvinyl lacto-phenol (A), fruitbody mounted in polyvinyl lacto-phenol (B) and spore print mounted in cotton blue solution (C).

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MICROPLATE TECHNIQUE OF PHYSIOLOGICAL TESTS FOR YEAST IDENTIFICATION

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Abstract. Yeasts are important in various industrial processes as producers or as contaminants. To identify yeasts quickly and reliably is crucial. Therefore exploring fast, simple and inexpensive identification methods is necessary.
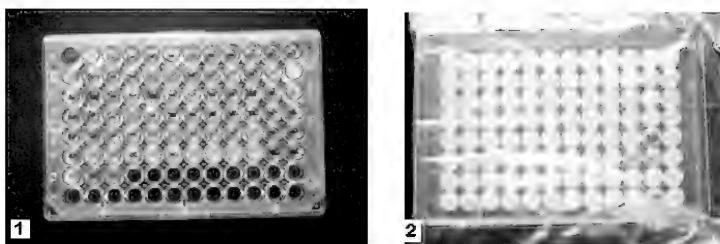
Fig. 1. Microplate 85 assimilative tests of one yeast strain. 2. Microplate grouping 12 fermentative tests of 8 strains in anaebiosis.
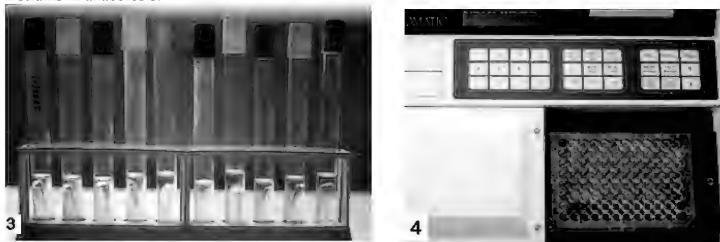
Fig. 3.Traditional optical reading of fermentative tests in tubes of one strain. 4. Automatic non-subjective reading of microplates of assimilative and fermentative tests.

Traditionally, yeasts are identified by morphological characters and physiological properties such as assimilation and fermentation tests according to the

procedure described by Van der Walt & Yarrow (1984) emended by Barnett *et al.* (1990).

Physiological tests using tubes are time consuming and expensive. We propose a new, fast and inexpensive technique for physiological tests using microplates. The oxydative microplate includes 48 carbone sources and 9 nitrogen sources for assimilation, 10 vitamines for minimum requirement, 3 cycloheximide and sodium chloride concentrations for tolerance, and the urease, the arbutin splitting and the starch formation tests. The fermentative microplate includes 12 carbon sources. The microplates are read automatically on a microplate reader. The absorbency measurements are transferred to a computer in a data matrix. A software has been created and programmed to interpret the absorbency measurement data in terms of positive, negative and weak results.

Together with morphological data, physiological data are then treated by the yeast identification expert system ALLEV for identification (Robert *et al.* 1994).

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CRYOPRESERVATION OF *ASPERGILLUS REPENS*

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Abstract. Cryogenic light microscopy has been used to develop optimum cooling protocols for fungi. The response to freezing and thawing of a fungus is demonstrated well by *Aspergillus repens* on cooling at -30°C min -1 to -30°C. Ice crystalization, solute crystalization and gas bubble formation cause injury to the fungus cell. Complete evacuation of cytoplasm from the hyphae has been seen.

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CULTIVATION OF THE FUNGI

ACTIVATED CHARCOAL-MEDIATED SPOROPHORE INITIATION IN *AGARICUS* AND *TRICHOLOMA*

ABED PEERALLY

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Abstract. Agaricus bisporus is known not to produce sporophore primordia in aseptic conditions unless activated charcoal is present. Using different techniques *Agaricus bisporus, A. bitorquis* and *Tricholoma spectabilis* were grown aseptically on distilled water agar, polyethylene foam, malt extract agar and soil with and without activated charcoal. Only *A. bitorquis* produced ill-defined primordia in the form of mycelial aggregates on water agar and malt extract agar in the absence of activated charcoal. In the presence of activated charcoal the three species produced primordia but only *A. bitorquis* and *T. spectabilis* produced normal pins and sporophores. The best fruiting was obtained with *A. bitorquis* on water agar treated with activated charcoal and with *T. spectabilis* on activated charcoal treated soil. It is concluded that all three species produce similar fruiting inhibitory factors capable of being neutralised by soil bacteria and activated charcoal.

Introduction

The usefulness of activated charcoal in stimulating the fruiting process of *Agaricus bisporus* (Lange) Sing. was initially reported by Mader (1943). This observation led Mader to conclude that volatiles were involved in the formation of carpophores. The stimulating effects of soil bacteria in the natural initiation of fruiting in *A. bisporus* was first observed by Eger (1961, 1972) and subsequently reliable additional observation on the bacterial induction of fruiting was made by Hayes et al. (1969), Hayes (1981) and Peerally (1978, 1981).

Eger (1961, 1972) also reported the ability of activated charcoal to replace the effects of bacteria. Similar results were also reported by various other workers (Long and Jacobs 1974, Convy 1976, Wood 1976, Peerally 1978, Stoller 1978).

That activated charcoal could also stilmulate fruiting in *Agaricus bitorquis* (Sacc.) Quel. was shown by Peerally (1978). However Stoller (1978) observed that on malt extract agar, activated charcoal and lignite separately had no effects on the initiation of fruiting in *A. bitorquis* but when mixed, fruiting was profusely stimulated.

It is generally believed that activated charcoal stimulates fruiting by removal of one or more inhibitors present in the mycelium. To date it is still not understood what precise role bacteria and activated charcoal play in carpophore initiation Ingratta and Patrick (1986) suggested that the major stimulus in carpophore initiation in *A. bisporus* might be related to the intensified microbial activity in the casing layer which results in a reduced nutritional base in the casing and an increase of fungistasis.

The present paper reports the fruiting response in *A. bisporus* (strain 133), *A. bitorquis* (strain 1) and in *Tricholoma spectabilis* Peerally & Sutra (local strain 1) under the influence of activated charcoal, in four different techniques.

Methods

Cultures were first obtaine on standard potato dewtrose agar (pH 7.5) and then used to onoculate autoclaved wheat grain in conical flasks. Grain spawn of *A bisporus* was incubated at 23°C while that of *A. bitorquis* and *T. spectabilis* was kept at 28°C. The three spawns were fully invaded and ready after about three weeks.

Plates of malt 2% agar (MEA) and double distilled water agar (DWA), both adjusted to pH 7.5 with sodium hydroxide and calcium carbonate, were prepared and after setting, the agar surface was thoroughly covered with a thin layer of activated charcoal powder (about 0.7g per plate). Plates were inoculated with single grain of spawn or with a small quantity of spawn.

Loam soil, pH adjusted to about pH 7.3 with calcium hydroxide, and polyethylene foam (8cm diam, 4mm thick) were placed in separate Petri dishes. Sterilisation of soil was achieved on three consecutive days for one hour. Plates with foam were autoclaved one for one hour. After sterilisation, activated charcoal powder was applied to the soil (0.3g or 1.0g per plate) and to the foam (1.0g per plate). Plates were then inoculated on one side with a small quantity of grain spawn, and sterile distilled water added.
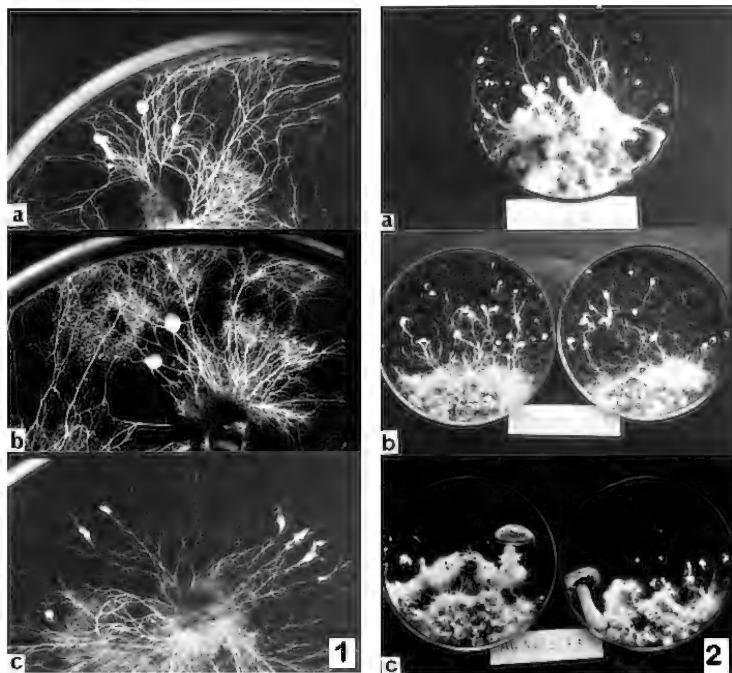
Fig. 1. *Agaricus bisporus* a-c Primordia production.
Fig. 2. *Agaricus bitorquis* a. On loam + activated charcoal. b. On soil + 0.3g activated charcoal. c. On soil + 1g activated charcoal.

They were at least ten replicates per treatment including the untreated control sets of plates. Experiments were repeated at least once.

Plates with *A. bisporus* were incubated for six days at 23°C and then transferred to an aerated room at 18-20°C. Plates with *A bitorquis* and *T. spectabilis* were incubated at 28°C for six days and then transferred to 21-26°C.

Results

Activated charcoal treated plates and control plates were observed daily. The state of mycelial growth and of fruiting initiation and development was studied over a period of two to four weeks. No attempt was made to count the number of primordia which for some treatments was indeed not feasible.

Colony growth in all untreated DWA plates was fluffy or slightly strandy. Primordia were not produced in *A. bisporus* and *T. specatabilis*. Some of the DWA plates with *A. bitorquis* produced carpophore initials but in a rather disorganized pattern, in contrast to organized, synchronous and regular development at the periphery of the colony obtained with activated charcoal. Those initials consisted of fuzzy hyphal aggregates (Wood 1978). Smooth-surfaced primordia and pins as produced in the presence of activated charcoal were never seen on untreated DWA. Initials were formed from day 7 or 8 after inoculation.

In DWA plates treated with activated charcoal colony growth was conspicuously adpressed with little aerial mycelium, in *A. bisporus* and *A. bitorquis*. Strandy growth sometimes occurred in *A. bisporus* and *A. bitorquis* conspicuously (Fig. 2a). In *T. spectabilis* strands were never observed, but instead isolated areas of fluffy mycelium were frequently produced (Fig. 1) from which well developed smooth primordia, pins and carpophores developed. Fully mature carpophores with stipe, pileus and gills were best formed by *A. bitorquis* and were profuse throughout the periphery of the colonies *A. bisporus* produced a few primordia (Fig. 3a-c) which did not develop further.

On MEA, *A. bisporus* and *T. spectabilis* did not produce any primordia whether activated charcoal was supplied or not. *A. bitorquis* produced a few mycelial aggregates but nothing further in presence of activated charcoal.

On foam without activated charcoal, mycelial growth of the three strains was very fluffy. In the presence of activated charcoal *A. bitorquis* produced very strandy mycelium, primordia, pins and mature carpophores (Fig. 2a). *A. bisporus* formed strandy mycelium but no primordia, while *T. spectabilis* grew to a limited extend and formed some primordias and pins.

In autoclaved soil treated with activated charcoal *A. bitorquis* (Fig. 2b, c) and *T. spectabilis* produced fluffy mycelial growth, primordia, pins and mature carpophores.

Discussion

Only *A. bitorquis* was capable of producing initials in the form of fuzzy myceliual aggregates on untreated DWA. In the presence of activated charcoal all three species produced pimordia, but only *A. bitorquis* and *T spectabilis* formed well developed smooth primordia, pins and carpophores, similar to those produced in normal casing and in nature, respectively. Only *T. spectabilis* showed the peculiarity of forming fluffy aerial mycelium capable of readily producing well developed pins and carpophores.
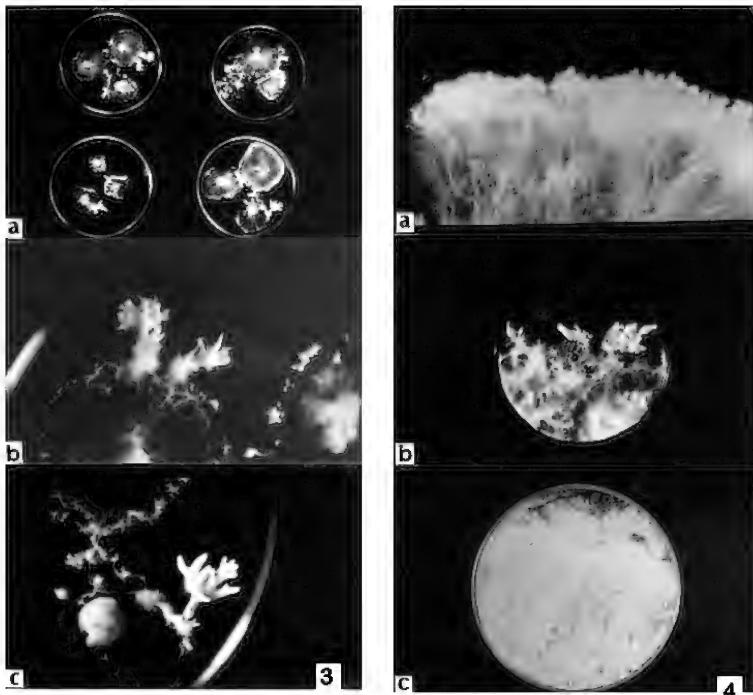
Fig. 3. *Tricholoma spectabilis.* a-c. fruiting on activated charcoal.
Fig. 4. *Tricholoma spectabilis.* a, b. mycelium and primordial on non aseptic soil. c. sterile mycelium on
    aseptic soil.

The fact that activated charcoal did not stimulated primordial on MEA supports
the results obtained by Stolla (1978)

San Antonio and Peerally (1978) working with several strains of *A. bitorquis*
concluded that there was no correlation between the formation of initials on agar
media and productivity under normal growing conditions. This might possibly be
attributed to the disactivation of activated charcoal by chemicals present in the malt
agar. It is, thus, conceivable that such a correlation might exist between formation of
primordias in the presence of activated charcoal and productivity of the strain.

The fact that all three species were capable of producing at least primordias in
the presence of activated charcoal is interesting. Bacteria are known to be necessary
for the formation of carpophores in *A. bisporus*, as well as for *A. bitorquis* and *T.
spectabilis* (Peerally unpublished) (Fig. 4a-c).

It must therefore be concluded that the same inhibitory factors are present in the
mycelium of these basidiomycetes and once neutralised or removed, fruiting takes

place. The induction of fruiting in widely different terricolous basidiomycetes by bacteria and activated charcoal could well have the same mechanism but the nature of this induction still remains to be elucidatedc.

Acknowlegement

My thanks are due to Mr V. Aumeer for technical assistance.

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IN VITRO CULTURE OF ENDOMYCORRHIZAL FUNGI: WHY THAT INTEREST?

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Introduction

Vesicular arbuscular mycorrhizal (VAM) fungi are found naturally in almost all tropical soils and under all terrestrial ecosystems (Sieverding 1991). Much of the interest in VAM fungi relates to their role in plant growth and phytoprotection.

Taxonomy of these fungi has been largely based on spore characteristics (Morton, 1988). Nevertheless, accurate identification of VAM fungi in field soils is not easy as the spores may be present in different stages of their development. Mixtures of spores of different species could also lead to identification problems (Abbott and Robson 1991). Taxonomy using biochemical and genetical characterizations has also been hampered by the lack of powerful techniques of specific and sensitive detection and obtention of DNA fragments in axenic conditions. Recently, the upsurge of the polymerase chain reaction (PCR) technology has given a new impetus in biomolecular research and has widespread applications in VAM fields as diverse as phylogeny, evolutionary history and taxonomy (Simon 1993).

Mosse and Hepper (1975) were the first to propose an *in vitro* system based on a dual culture of spores and excised roots. Mugnier and Mosse (1987) and Becard and Fortin (1988) improved the system by using roots genetically transformed by *Agrobacterium rhizogenes*. Diop *et al.* (1992) were able to follow the entire vegetative cycle of *Gigaspora margarita* using Ri-TDNA transformed carrot roots. Another method of investigation was proposed by Strullu and Romand (1986, 1987 and 1991) who developed a culture technique based on the intraradical phase of the fungus i.e. the vesicles.

The continuous culture of VAM fungi means that the mycorrhizal fungi must be indefinitively subcultured in order to maintain and increase its biomass. Becard and Fortin (1988) achieved continuous cultures of *G. margarita* using the spores produced *in vitro* by this VAM fungus. Strullu and Romand (1986) proposed a method using mycorrhizal root as starting material and based on the successive reassociation of mycorrhizal roots with non mycorrhizal root systems. This system of *in vitro* subcultures has been applied successfully by these authors with *Glomus* species.

The indefinite *in vitro* maintenance of VAM fungal species would make it possible to maintain biodiversity through the creation of fungal libraries. Taxonomy based on morphology and molecular biology (PCR) should also lead to the characterization of these species which should become "reference species".

We propose an application of *in vitro* culture using the intraradical phase of the VAM fungi, applied to banana in order to obtain appropriate strain for sustainable agriculture.

Methodology

Sampling, trapping and isolation procedures

Infective VAM fungal propagules are collected in the field by soil and root sampling (1)[*]. Soil samples are taken in the upper layer of the soil where most root growth occurs. The indigenous VAM population present in soil and roots were then multiplied and maintained on trap plants (*Allium porum*). The procedure of trap pot culture is simple (2). Pots are filled with calcined clay (Terra Green) on top of wich the field soil or root sample is placed. Another layer of Terra Green is placed above the soil or root sample. A high mycotrophic plant with good growth and rooting e.g. leek, is then planted in the pot. After several months, soil and root samples are taken and screened for mycorrhizal infection. Soil and root material is then suspended in water and after 10 - 15 s (for sedimentation of coarse sand) the suspension is decanted over a series of soil sieves (2mm - 500 - 250 - 125 - 50 microns). Roots in the top sieve are washed under tap water and transferred to a petri dish. Infected roots are further separated from the noninfected roots under the stereomicroscope (3). Infected roots are conserved at 4°C. Spores (4) are isolated from the sieves and

used as starting material for monosporal culture on leek plants (4'). Spores are also used for taxonomic description of the field isolates (4"). Collections of the VAM isolates are maintained by using the sieved soil to establish pot culture (5).
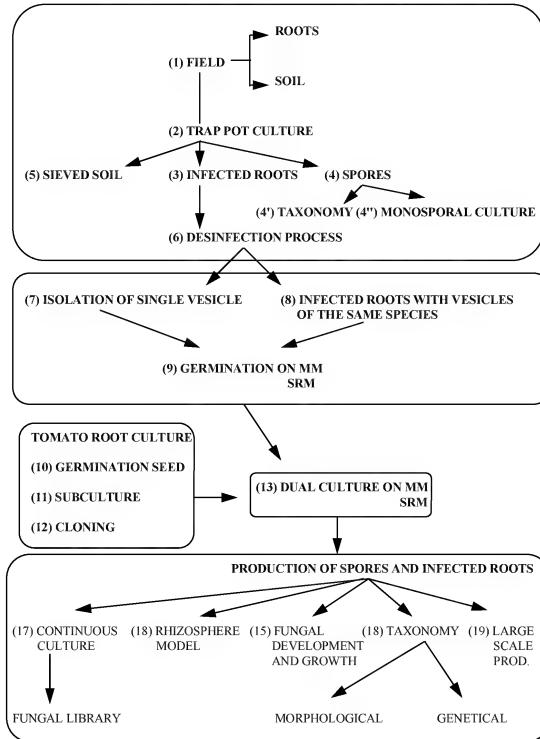
In vitro culture
Fungal inocula

Infected roots containing vesicles are desinfected using the procedure developed by Strullu and Romand (1986) (6). The roots are surface sterilized succesivelly with 95° alcohol for 10 s, 6 % Ca hypochlorite for 2 min, 2 % chloramine T for 10 min and 200 mg/l streptomycin - 100 mg/l gentamycin for 10 min. Roots are rinsed with sterilized water after each sterilization step. Sterilized roots are conserved in Streptomycin (200 mg/l) - gentamycin (100 mg/l) at 4°C until ready for use. Vesicles (7) are extracted from roots and germinated on Minimal (Becard and Fortin, 1988) or on Strullu -Romand (1986) medium (9). Roots containing vesicles of the same species (8) are cut in pieces of 0.5cm and also placed on the same mediums as described above (9).

Root organ culture

Initiation of root culture requires seed germination under axenic conditions. Emerging radicles are then excised and cultured on White's medium (White 1943). Tomato seeds are surface sterilized by soaking in 15 % hydrogene peroxide for 2 - 3 minutes. Seeds are then rinsed in sterile water and germinated (10) in the dark in sterile Petri dishes on water - agar. When radicles are 2 cm long, they are excised and transferred onto White's medium (White 1943) (11). Roots with the most laterals are then chosen to initiate clonal culture (12).

Dual culture

Dual culture of root and fungus is established on Minimal medium (Becard and Fortin 1988) or on Strullu - Romand medium (1986) (13). Pre-germinated single vesicles or mycorrhizal root pieces, containing vesicles of the same species, showing hyphal regrowth are used to inoculate the root system by placing them close to emerging lateral roots. Extra and intra-radical development of the fungus is eventually obtained depending on the fungus, the host and the environmental conditions.

Conclusion

The root organ culture technique for VAM fungi has numerous advantages over traditional inoculum production systems. It allows large scale production of inoculum free of pathogens (14). It permits reproducible observations of all stages of VAM fungi development (15) and can be used as a simplified rhizosphere model (16) for ecological studies i.e. effect of heavy metal toxicity, competition with other microorganisms, interactions with plant root pathogens, pesticides... The system is also useful for determining symbiotic factors provided by the root which govern fungal growth (CO_2, root exudates...) (16). The continuous culture of VAM fungi would make it possible to maintain biodiversity through the creation of fungal libraries (17). Taxonomy based on morphology and molecular biology (PCR) should also lead to the characterization of these species which should become "reference species" (18).

Development of pure culture of endomycorrhizal fungi continues to represent one of the most challenging goals in fungal biology. It is accepted that understanding the mechanisms involved in the symbiotic process will lead to the development of pure culture in the near future.

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ENHANCEMENT OF CARPOPHORE PRODUCTION AND PROTEIN CONTENT OF *PLEUROTUS SPP.* BY CULTIVATION ON GRASS HAY.

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Abstract. Seven strains of *Pleurotus* mushrooms (*Pleurotus ostreatus* var. *columbinus* MUCL 28785, *Pleurotus ostreatus* var. *columbinus* MUCL 30495, *P. cornucopiae* MUCL 31019, *P. ostreatus* var. *ostreatus* cv. *Florida* MUCL 31018, *P. pulmonarius* MUCL 28784 and *P. sajor-caju* MUCL 31017) were cultivated on grass hay and wheat straw without additives. The grass hay increases both production of carpophore and protein content of all strains.

Stimulating factors of grass hay was identified by supplementing wheat straw with selected and combined chemical compounds.That study was conducted with *Pleurotus ostreatus* var. *columbinus* MUCL 28785 cultivated on all supplemented susbstrates. Several compounds (alanine, asparagine, biotin, gibberellic acid, glutamic acid, glycin, indole-acetic acid, indole-butyric acid, kinetin, manganese and serine) have been able to enhance carpophore production. The main stimulating factor of grass hay was the interaction between amino acids and manganese.

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IS THE RUST OF THE CREEPING THISTLE (*CIRSIUM ARVENSE*) THE MICROSYMBIONT OF ITS VAM ?

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Abstract. This paper investigates the causing factor of growth of the creeping thistle (*Cirsium arvense*) as representative of a group of plants which really bears the qualities of weeds as it can crop up anywhere and cannot be controlled by normal soil cultivation methods. On the other hand they often do not endanger the yield of the crop. *Rumex crispus, Agropyron repens, Convolvulus arvense* and *Equisetum arvense* belong to this group of weeds. After use of pesticides and fungicides the problem subsists. The failing control of the creeping thistle shows a very unsatisfactory theory of its growth. Growing factor and sprouting are obviously the same. The thistle has a VAM. Rhythm of growth and physiologic conditions of

the soil are similar to those of orchids. Sprouting from the seed does not show cotyledons but a whole plant.

The invincibility of these weeds obviously rests on an assimilatoric contribution by dissimilation in the soil.

Some Orchidaceae, Convolvulaceae and Scrophulariaceae posses VAM. In the wild a regular vegetal association of the creeping thistle can be found with orchids such as *Epipactis helleborina* and *Platanthera bifolia*, and Convolvulaceae such as *Convolvulus arvense*. The question arises whether exists a similar symbiotic contribution to thistle growth, also in agricultural systems.

The ecologic niche of orchids can be destroyed easily by stress on the soil microbiology e.g. by action of fertilizers or other chemicals, fungicides or pesticides. The creeping thistle is also sensitive but temporally.

The best method of creeping thistle regulation are legumes in crop rotation. Red clover (*Trifolium pratense*) or alfalfa (*Medicago sativa*) obviously have an antagonitic effect on the thistles. The action maybe due to stem rot by *Sclerotinia trifoliorum*, but the attacked patches in the field are very soon subjected again to growth of thistles, *Agrpyron repens* and/or *Rumex crispus*.

The root of *Cirsium arvense* can have a phase of dormancy for many years, this means that roots survive in soil but do not emerge. Also thousands of seeds are waiting in the soil. Do both are waiting for the action of a microsymbiont, like do species on Orchidaceae? Indeed the author interprets the fact as the possible existence of a comparable mycothrophy by VAM.

Creeping thistle is associated with a number of fungi, foliar pathogens such as rusts *Puccinia suaveolens* or *Albugo trapognonis*, Coelomycetes *Septoria circii* or *Phyllosticta cirsii*, stem fungi like *Leptosphaeria* or *Ophiobolus* spp. or root attack by nematodes. But none of these organisms is known to enhance growth of thistles. Then an unidentified VAM or endophytic fungus might be responsible of the weed survival and might provide the key of its control.

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MORPHOLOGICAL STUDIES OF MYCELIA FROM ECTOMYCORHIZIAN FUNGI CULTIVATED *IN VITRO*

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Abstract. Different ectomycorhizian fungi belonging at different genus were put in culture from carpophore fragments. Morphological studies of mycelium growing on different culture media were described. Chemical reactions obtained on in vitro mycelium are compared with results obtained on carpophore.

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FUNGAL BIOTECHNOLOGY

YEAST FUNGI AND ALCOHOL FROM BAMBOOS

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Introduction

The bamboo *Oxytenanthera braunii* is used by the local people in southwestern Tanzania, to obtain an alcoholic beverage locally known as "*ulanzi*" (IBRN 1993). This is attained through fermenting sugary exudates, from young, cut and bruised shoots of the bamboo, which come out during the rainy season (Kigomo 1990). The sugary exudates are made to flow into a collecting container known as "*mbeta*" (Fig. 1 & 2). Exudates from many "*mbeta*" are then pooled together in 20-liter plastic containers, brought into a cool corner of a house and left to ferment.

The fermentation of the bamboo exudates is a wild and natural one, as no special fermenting microorganisms are purposely inoculated into the bamboo sugary exudates. The beverage, reaches its maximum alcohol amount within 24 hours. Obtainage of an alcoholic beverage from bamboos, is practised only in the southwestern areas of Tanzania, and no where else in the world.

So far, microorganisms involved in the fermentation of the bamboo sugary exudates, have not yet been documented. This work was aimed at isolating microorganisms involved in fermenting sugary exudates of the bamboo to alcohol; and also to quantify amounts of alcohol produced.

Materials and methods

Slants of sterile medium in MacCartney bottles were brought to a bamboo growing area in southwestern Tanzania, for initiating isolation of the fermenting microorganisms. An initiation of isolation of microorganisms from plant exudates is best done on the surface of agar-containing medium (Carmo-Sousa 1969). The agar medium in the McCartney bottles was a Wickerham maintenance agar medium (Phaff *et al.* 1978) containing yeast extract (0.3%), glucose (1.0%), malt extract (0.3%), peptone (0.5%) and agar (2.0%).

While working in a kitchen at the bamboo growing area, a wire loop was sterilized by heating it to red hot on a spirit lamp, and cooled in absolute alcohol. On cooling to ambient temperature, the wire loop was used to take samples from sterile membrane filters which had just been used to filter-sterilize fresh bamboo exudates for other studies. The contents on the wire loop were striken on the slants in the McCartney bottles. The bottles were then properly closed and brought to the laboratory for purification and identification of the prevalent fermenting microorganisms (Lodder 1970).

Several aliquots of fresh bamboo exudates 900 ml in volume, were hygienically also collected in pre-sterilized 1 liter bottles with butyl rubber stoppers and metallic caps. Two sterile hypodermic needles were injected on each bottle through the rubber stoppers, to release possible CO_2 that could be produced during transportation. The bottles were immediately placed in well protected ice-boxes and transported to reach the laboratory in Dar es Salaam, the same day. In the laboratory, the exudates were fermented naturally, at room temperature ($28 \pm 1°C$).

Fig.1. A stand of bamboos, having many "*mbeta*" hung on various young, cut bamboo shoots
Fig.2. Close up of a yound, cut bamboo shoot with a "*mbeta*" hung on it for collection of exudates

Samples were taken from the fermenting set-ups on daily basis for determination of types and amounts of alcohols in them on a Gas-Chromatograph (Magingo and Gijzen 1989).
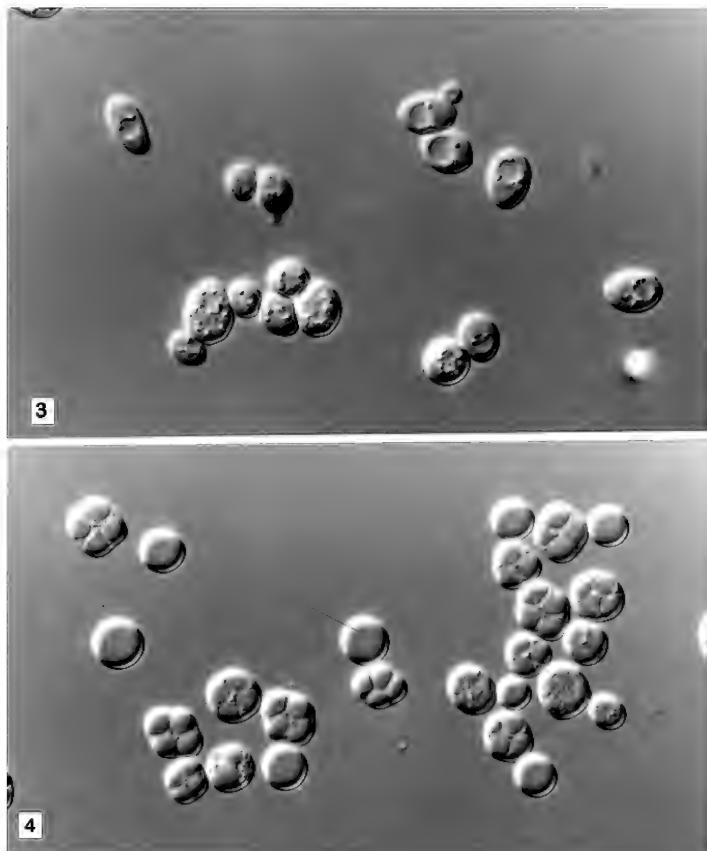
Fig.3. Asexual cells of the *Saccharomyces* sp. From bamboo exudates
Fig.4.Cells of the isolated *Saccharomyces* sp., some having ascospores

Results and Discussion

A *Saccharomyces* species (Fig 2-3) was the most prevalent microorganism fermenting the bamboo sugary exudates. The isolated yeast could also ferment glucose, galactose, sucrose and raffinose. The yeast isolated, could ferment 27% w/w pure glucose, to produce and withstand 9.5% w/w ethanol.

In terms of alcohols, only ethanol was detected in the fermented bamboo exudates taken from one village in Iringa, Tanzania. The alcohol production reached 6.5% w/w on average. It was also established that, at the sampling village, one stand (clump) of the bamboo has an average of 15 young shoots, each actively producing an average of 800g of the sugary exudates in one day. Such a clump, occupies an

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average area 3m in diameter. It thus follows that one stand of the bamboos can produce 12 Kg of the sugary exudates in a day which, on fermentation, will have 0.78 Kg or 1 liter of pure ethanol. If a 2m inter-stand space is allowed in a hectare of the bamboo, there will be 400 stands of the bamboo in the hectare, producing a total of 312 Kg of pure ethanol in a day. In other words, 400 liters of pure ethanol can be produced by the bamboo, per hectare, per day. If such alcohol is distilled by solar-powered equipment, it might be a cheap source of ethanol for various uses.

The present isolated yeast from bamboo exudates, could withstand 9.5% alcohol. It is proposed here that, further work on the bamboo fermenting microorganisms, could be directed at searching for other microbial strains or types in the bamboo exudates, which can ferment higher concentrations of sugars, and/or those which can withstand higher amounts of alcohols.

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GROWTH OF *FUSIDIUM COCCINEUM* ON SOLID MEDIA

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Abstract. Growth of Fusidium coccineum on both complex and defined agar media resulted in the production of fusidic acid into the solid medium. Production of the antibiotic was found to be growth associated in both cases with production ceasing on depletion of glucose. Conidiation did not occur in any of the cultures examined indicating the absence of a temporal link between antibiotic production and conidiation in this organism.

Introduction

Fusidic acid is a clinically important antibiotic that inhibits protein synthesis at the level of translocation in both prokaryotes and eukaryotes (Tanaka *et al.* 1968). It exhibits potent activity against gram-positive bacteria (Godtfredsen *et al.* 1966) and is used extensively in the treatment of staphylococcal infections in either cream or tablet form.

Fusidic acid is produced by conventional submerged fermentation using the fungus *Fusidium coccineum* (Von Daehne *et al.* 1984). Despite the obvious clinical importance of this metabolite little information has been published on the physiology of the producing organism, for a review see Von Daehne *et al.* (1984). This organism differentiates in both surface and submerged culture and a link between conidiation of the fungus and fusidic acid production has been reported,

(Navashin *et al.* 1981). To study this relationship further we examined the growth of the organism on agar solidified media.

Material and Methods

Organisms

Fusidium coccineum (CBS 197.55) was used throughout the study. This was maintained by weekly subculture on YEPG agar and incubation at 28°C. *Corynebacterium xerosis* (NCTC.9755) was used in the bioassay protocol and was maintained by subculturing on CX agar slopes with incubation at 37°C.

Media

YEPG medium contained, per litre: 10g yeast extract (Difco), 20g glucose, 10g mycological peptone (Difco), 20g agar. Liquid starter cultures, 50ml in a 250ml conical flask, were prepared using the above medium without agar, followed by inoculation with a 5mm diameter plug from a 7 day old YEPG plate. Cultures were incubated in an orbital shaker (200 rpm), at 30°C for 7 days. Aliquots (100Êl) of the liquid cultures were used to inoculate plates of YEPG agar. Each plate was prepared with a cellophane membrane overlay and confluent growth was produced by spreading the inoculum across the surface of the cellophane. Plates were incubated at 30°C for various times as shown. Minimal medium contained, per litre final concentration: 10g glucose, 3.36g sodium dihydrogen orthophosphate (dihydrate), 0.85g sodium nitrate, 0.75g potassium chloride, 0.64g sodium sulphate decahydrate, 0.26g magnesium chloride hexahydrate, 2.2mg calcium chloride, 24Êg sodium molybdate dihydrate, 0.292g EDTA. Trace salts. 2.0mg zinc oxide, 27mg ferric chloride hexahydrate, 10mg manganese chloride 0.85mg cupric chloride dihydrate, 2.4mg cobalt chloride hexahydrate, 0.3mg boric acid and 20Êg biotin. The pH of the medium was adjusted to 6.8 with 1M NaOH prior to sterilization. The trace salts solution was made up separately as a hundred fold concentrate, the pH was adjusted to 2.0 with concentrated hydrochloric acid and sterilised at 121 C for 15min. Glucose was also sterilised separately as a ten fold concentrate.

For the physiological experiments 20ml of medium was dispensed into each plate and allowed to solidify prior to placing a pre-sterilised cellophane membrane on the surface. Cultures were inoculated directly on the surface of the membrane and spread with a glass spreader to achieve a confluent inoculum.

Analysis of samples

Biomass was determined by dry weight after removal of cell material from the surface of the membrane with a razor blade. The biomass was placed on a Whatman Nº1 filter and dried in a microwave oven for 15 min. on the "defrost" setting.

Samples were taken from beneath the membrane using a cork borer (5mm diam.). The agar plugs were then subjected to a freeze squeeze protocol to provide liquid medium samples using the method of Tautz & Renz (1983). Glucose was measured using a glucose oxidase kit (Boehringer-Mannheim). Fusidic acid was measured by bioassay using *C. xerosis* (Bywater 1975).

Results
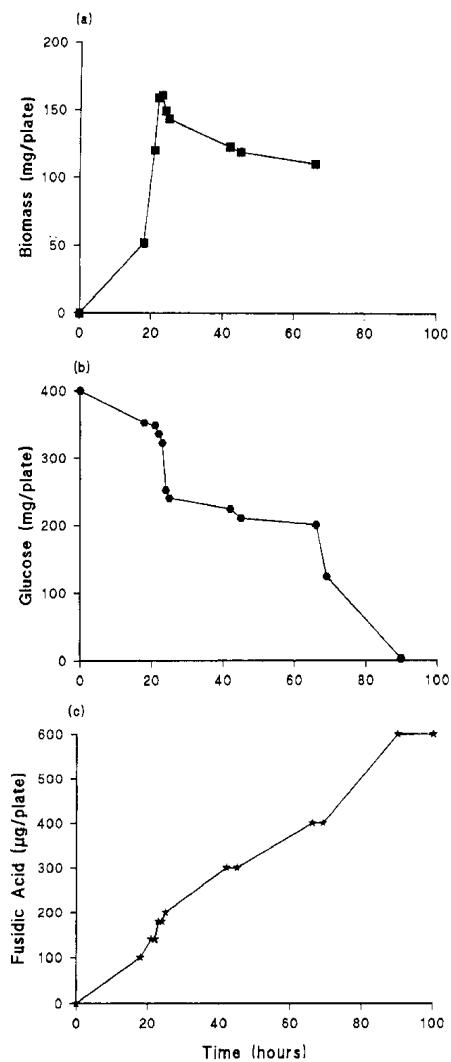
Fig.1. Growth of *F. coccineum* on YEPG medium. (a) biomass, (b) glucose, (c) fusidic acid. Values represent the mean of at least three determinations.
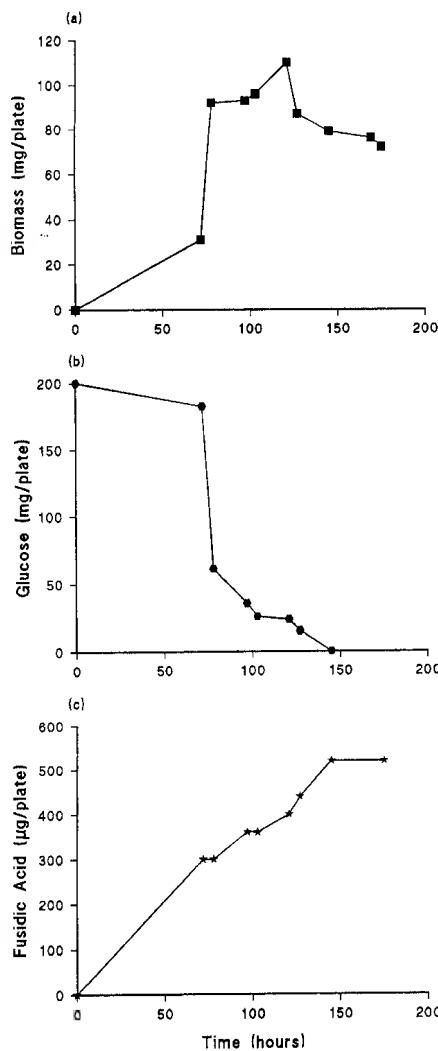
Fig.2. Growth of on minimal medium. (a) biomass, (b) glucose, (c) fusidic acid. Values represent the mean of at least three determinations.

Growth of *F. coccineum* on complex medium was rapid with maximum biomass levels being achieved within one day of inoculation (Fig 1a). The biomass accretion coincided with the consumption of glucose from the medium (Fig

1b).Approximately half the available glucose was consumed during this initial growth period. After 21 hours of growth, biomass levels declined possibly due to nutrient limitation or diffusion limitation. Measurements of fusidic acid concentrations in the medium revealed a biphasic production pattern, with an exponential increase in antibiotic during the growth phase followed by a linear accumulation after biomass accumulation had ceased (Fig 1c). Glucose levels throughout the experiment were found to decrease, reaching an undetectable value after 90 hours post-inoculation. The complete removal of glucose from the medium resulted in the cessation of fusidic acid accumulation, suggesting an intimate link between antibiotic production and glucose availability. The cultures were monitored throughout for the appearance of morphological differentiation, particularly for the appearance of conidia. At no time during the experiment were conidia observed suggesting that under these conditions at least, no link between antibiotic production and conidiation could be shown.

To further investigate the relationship between glucose availability, conidiation and antibiotic production we developed a defined minimal medium that would support growth and antibiotic production. Growth of the culture on minimal medium was slower than for complex medium, reaching maximum biomass after 120 hours (Fig 2a). Biomass accumulation ceased close to the point of glucose exhaustion suggesting that growth in this culture was probably glucose limited (Fig 2b). Fusidic acid production was growth linked under these conditions and as with the complex medium the production of the metabolite ceased at the point when glucose completely disappeared from the medium (Fig 2c). Examination of the cultures for evidence of conidiation revealed a complete absence of conidia throughout the experiment, providing further evidence for the lack of association between conidiation and antibiotic production in this organism.

Discussion

Plate cultures provide a simple and convenient way of investigating the connection between morphological differentiation and metabolite consumption and production. The evidence provided herein suggests that conidiation and fusidic acid production are physiologically separate entities. This data conflicts with earlier work of Navashin et al.(1981) who describe a temporal link between conidiation and antibiotic synthesis in *F. coccineum*. Furthermore, our work would also suggest that the cells responsible for the production of the antibiotic are the hyphal form rather than the conidia themselves. The kinetics of production of the antibiotic on solid medium is not typical of a secondary metabolite with the greatest rate of production occurring during the rapid growth phase. The question then arises as to whether fusidic acid is an "overflow metabolite" of primary metabolism or a true secondary metabolite.

Clearly fusidic acid production is dependent upon available glucose, an efficient protocol for production therefore may be to use immobilised resting cells of the fungus which could be fed continuously with glucose.

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STRAIN POLYMORPHISM IN PDR1, A TRANSCRIPTION REGULATOR OF PLEIOTROPIC DRUG RESISTANCE GENES IN *SACCHAROMYCES CEREVISIAE.*

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Abstract. PDR1 is a Zn2 Cys6 transcription factor that regulates positively PDR5 and probably other mutidrug pumps in *Saccharomyces cerevisiae.* (Balzi et al. 1994).

The comparison of the PDR1 DNA sequence has been carried out on the following seven so called wild type laboratory strains: IL 125-2B, D 286-2A, FM 11, GR 350, JG 200, FY 1679-28C, S288C.

Seven nucleotidic differences clearly distinguished the strains of european origin (IL1252B, D 286-2A) from those of american origine (JG200, GR 350, FM11, FY 1679-28C, S288C). Four out of the seven differences resulted in aminoacid changes, and three others were only at the nucleotide sequence. In all european strains, the amino acid residues 411, 821 and 921 were found to be lysine, alanine and isoleucine instead of glutamine threonine and threonine respectively in the american strains. The fourth difference was observed in the polyasparagine strech beginning at residue 1009: in the european strains five successive asparagine residues were observed instead of ten asparagines in the american strains.

Thus the polymorphism is more pronounced in the C-terminal region of the protein.

In this family of transcription factors the C-terminus is usually involved in transactivation of transcription whereas the N-terminus bears the zinc finger domain which binds DNA on specific consensus motif. We will construct a set of a isogenic strains carrying the different wild type alleles to investigate whether or not these modifications affect the transactivation activity of PDR1.

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STRUCTURAL IDENTIFICATION AND BIOCHEMICAL MODE OF ACTION OF A MYCOTOXINE ISOLATED FROM *COCHLIOBOLUS SATIVUS* (MUCL 18528)

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Abstract. A mycotoxin extracted from the culture medium of *Cochliobolus sativus* has been purified by chromatography (TLC, HPLC) and identified by infrared, mass and NMR spectrometry. The chemical structure corresponds to the "helminthosporol", a sesquiterpene discovered in 1965 by S. Tamura.

Our results on the biochemical activity show that this compound inhibits the oxidative phosphorylation of plant mitochondria (ATP synthase) but does not inhibit the ATP phosphohydrolase (ATPase) activity. In contrast the mitochondrial ATPase activity of the yeast *Saccharomyces cerevisiae* is strongly inhibited by the toxin. The plasma membrane ATPase activity of *Saccharomyces cerevisiae* is not inhibited by helminthosporol.

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CHARACTERIZATION OF THE CARBOHYDRATE MOIETY ON PROTEINS SECRETED FROM DIFFERENT FUNGI

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Abstract. Many different yeasts and filamentous fungi are attractive candidates for the production of secreted, therapeutically valuable proteins. Since the glycosylation pathway in these lower eukaryots differs from that in mammalian cells, oligosaccharides different from the complex, mammalian type are synthesized. Fungi-type glycosylation, known to be mannan or (for certain species) galactomannan forms an obstacle to application of heterologous proteins as therapeutic agents. The latter proteins are rapidly cleared from the blood because of recognition and binding of fungi-like oligosaccharides to sugar binding receptor proteins (Summerfield et al., 1986). With the exception of *Saccharomyces cerevisiae,* detailed knowledge about glycosyl structures is very poor. Yeasts are known for their capacity to hyperglycosylate proteins. Filamentous fungi, on the other hand, seem to synthesize smaller glycans (Berka, R.M. and Barnett, C.C, 1989). This resembles more the situation in mammalian cells.

With the aim to obtain more information about the diversity of oligosaccharide-structures and about the possible existence of glycosyl structures more similar to the mammalian system, we studied glycoproteins from a collection of yeasts and filamentous fungi. Lectins were used to investigate the identity of terminal sugar residues different from mannose. In a collection of yeasts, a few species seemed to have terminal a-linked galactose residues: Binding of *Bandeiraea simplicifolia* I

lectir, highly specific for terminal a-linked galactose, to their secreted proteins, was demonstrated. We are now investigating a collection of filamentous fungi, to determine whether new glycosyl patterns can be found.

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SPECIFIC AND NON-SPECIFIC INTERACTIONS IN YEAST CELL FLOCCULATION

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Abstract. At the end of the fermentation in brewery, cell aggregates are formed which accumulate on top of the fermented wort (top strains) or which sediment to the bottom (bottom strains). The aim of this research is to understand better the mechanisms which control the yeast aggregation, in particular the importance of long range interactions (double layer interactions, van der Waals forces), short range non-specific interactions (hydrophobic interactions, salt bridges) and specific interactions (molecular recognition).

The yeast surface chemical composition is determined by X-Ray Photoelectron Spectroscopy (XPS); the hydrophobicity is evaluated by contact angles and the surface electrical properties are characterized by the zeta potential. The tendency of the cells to form aggregates is determined by turbidimetric measurements after controlled stirring and subsequent settling in defined solutions.

Top fermentation strains have been found to be more hydrophobic, to have a lower phosphate surface concentration and a less negative zeta potential. A more detailed comparison has been performed between one top and one bottom strain. For both strains aggregation takes place only at the end of the exponential growth phase and it requires the presence of calcium. The tendency of the top strain to form aggregates is maximum between pH3 and 4.5, i.e. close to its isoelectric point of 4. For the bottom strain the isoelectric point is much lower than the optimum aggregation pH range: however the decrease of the phosphate surface concentration during the culture is in accordance with an influence of electrostatic interactions. In the adequate range of pH, flocculation of the bottom strain requires addition of about 0.1 mM Ca^{++} while the top strain flocculates without calcium addition. The two strains exhibit also different responses with respect to the addition of organic compounds in the solution. For the bottom strain, certain sugars provoke an inhibition of flocculation or a redispersion of previously formed flocks; this is a specific effect, occurring with 10 to 500 mM glucose, mannose and maltose, but not with galactose, which points to the involvement of molecular recognition in cell aggregation. Flocculation of the top strain, on the contrary, is not influenced by these sugars; furthermore, addition of 5% ethanol broudens considerably the pH

range (2.5-5.5) in which flocculation takes place suggesting that solvation forces play an important role.

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MODIFICATION OF SUPPORT SURFACE PROPERTIES TO IMPROVE MICROORGANISM ADHESION

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Abstract. For immobilisation of fungi in bioreactor, a quick adhesion and homogenous distribution of the spore inocculum on the support surface is desirable. This is expected to lead to a more uniform growth and colonisation of the support.

Polycations and ferric ions were adsorbed on the surface of a flat polycarbonate support, either as such, or pretreated by sulpho-chromic mixture. The pretreatment grafted sulphate or sulphonate groups, which lowered the isoelectric point and rendered the surface more negative. The adsorption of polycations and ferric ions increased the zeta potential and isoelectric point of these surfaces. 5-10 atomic layers of adsorbed polycations were found at the pretreated support surface; on the non-pretreated supports, less than one atomic layer was adsorbed.

The supports were then submitted to a flow of *Phanerochaete chrysosporium* spore suspension; these fungal spores have a hydrophobic and negatively charged surface. A few spores adhered in a patchy way on the native supports. Adsorption of polycations and ferric ions increased the amount of adhering spores and led to a homogenous distribution. This might be due to the reduction of the electrostatic repulsion between the spores and the support, which increased the chance for the spores to come in contact with the surface and to adhere.

The spores adhering to the supports were later submitted to a flow of culture medium. Neither adhesion on the support, nor the presence of adsorbed polycations inhibited or delayed the swelling, the gerrnination and the growth of the spores.

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BACTERIOLOGY

GAS CHROMATOGRAPHIC ANALYSIS OF CELLULAR FATTY ACIDS IN THE IDENTIFICATION OF MICROORGANISMS

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Since Abel *et al.* (1963) demonstrated that fatty acid composition can be useful to classify microorganisms into genera and species, various workers have successfully used it to characterize, differentiate and classify bacteria.

As the cellular fatty acid profile is affected by a number of parameters, rigid standardization of experimental conditions and techniques is required. The extraction and derivation procedure proposed by Miller and Berger (1985) is commonly used in bacterial analysis. It consists in the main following steps:

1. saponification (NaOH in MeOHaq, 100°C, 30 min.)
2. methyation (HCI/MeOH, 80°C, 10 min.)
3. extraction (hexane/ethylether, 10 min.)
4. base wash (dilute NaOH, 10 min.).

The advantage of this method are the low time consumption, the low volume of reagents (each 1 to 2 ml) and the quantity of bacterial material require (about 40mg).

We applied this procedure to about 300 foodborne bacteria, grown under standardized conditions. The reproducibility, from bacterial cultivation to the GC analysis, was estimated at So=94.20±2.80 (n=84), where So is the overlap coefficient (Bousfield *et al.* 1985). Inside a species, the So was always greater than 79.

The great diversity of bacterial fatty acids, including saturated, monounsaturated, straight chain, branched-chain, 2- and 3-hydroxy and cyclopropane types, and the variability of their relative proportions permit one to define arge groups of strains (lactic acid bacteria, *Enterobacteriaceae*,...) and to separate closely related genera (*Micrococcus, Staphylococcus*) (Table 1).

The same extraction and derivatization procedure was also applied to characterise yeast strains. It should be noted that this procedure does not allow one to separate fatty acids from long-chain hydrocarbons, which elute after fatty acids in GC analysis.

Four stains of *Saccharomyces* (*S. bayanus* MUCL 31495; *S. pastorianus* MUCL 31496; *S. cerevisiae* MUCL 31497; *S. paradoxus* MUCL 31498) were investigated after grown for 72 hours, at 25°C, in dextrose 20g/l, yeast extract 5g/l, meat peptone 10g/l broth, on a rotary shaker at 160 rpm.

The profiles obtained were rather simple with a preponderance of saturated and monounsaturated C16 and C18 acids (Table 2).

In addition, in certain genera (*Bacillus, Pseudomonas*), the profile So obtained may be used as characteristics in speciation (Fig. 1).

Table 1. So value calculated between different bacterial genera.

	N°	1	2	3	4	5	6	7	8	9
Gram - Enterobacteriaceae										
Escherichia coli ATCC 10536	1	-								
Salmonella RL	2	92	-							
Pseudomonas fluorescens UPB 408	3	71	75	-						
Gram+										
Bacillus subtilis ATCC 12711	4	3	3	4	-					
Lactic acid bacteria										
Lactobacillus plantarum DSM 20174	5	57	55	46	4	-				
Leuconostoc mesenteroödes NCIB 3354	6	56	57	47	5	89	-			
Lactococcus lactis LMG 6897	7	40	39	29	5	70	65	-		
Pediococcus acidilactici NCIB 6990	8	61	58	52	3	74	69	66	-	
Staphylococcus aureus MBLA 210	9	4	4	5	73	5	7	6	5	-

So

Fig.1. Dendrogram of *Bacillus* species based on the So values.

Table 2. Fatty acid composition of *Saccharomyces* strains (% total fatty acids)

Strain	Fatty acid							
	12:0	14:0	16:0	18:0	14:1(9c)	16:1 (9c)	18:1 (9c)	18:1 (11c)
S. bayanus MUCL 31495	1,37	0,59	9,51	3,22	0,78	61,18	19,69	0,01
S. pastorianus MUCL 31496	1,56	0,70	2,42	0,24	2,32	77,25	14,28	0,20
S. cerevisiae MUCL 31497	2,33	0,81	3,32	0,74	3,64	63,04	23,50	2,26
S. paradoxus MUCL 31498	0,83	0,93	5,98	0,45	1,48	68,28	19,78	0,95

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The four strains had palmitoleic acid as the major component with an occurrence of up to 76% of the total fatty acid. The proportion of oleic acid varied from 14 to 27% among strains. The palmitic acid is several folds more abundant than stearic acid. Saturated and monounsaturated C12 and C14 were present in lower proportions. Traces (< 1%) of 15:1, 15:0, 16:1 (11c) and 17:1 acids were observed. No branched-chain and cyclopropane acids, which are common components of certain bacterial lipids, appeared in the profiles.

The qualitative composition is in agreement with previous reports on *Saccharomyces* strains (Augustyn and Kock 1989, Kock *et al.* 1986, Moss *et al.* 1982) and is highly similar to those found for other yeast genera (Moss *et al.* 1982, Smit *et al.* 1987). Therefore, the relative simplicity of the fatty acid composition of yeasts does not seem to offer as much aid in the taxonomy of the unicellular fungi as it does for the bacteria. Betwee the four strains studied, So was greater than 82, which is a higher value than for bacterial intra-species similarity. This does not allow one to draw conclusions on the classification of these strains in one or different species. Further experimental work is needed on more strains to evaluate the ability of using quantitative differences to composition to characterise, classify and identify yeast strains.

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IDENTIFICATION OF LACTIC ACID BACTERIA USING RAPD-PCR.

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Abstract. Lactic acid bacteria play an essential role in food technology. They improve the aroma and texture in food, and inhibit the growth of spoilage bacteria. However, some strains of lactic bacteria are involved in food spoilage and few are found even pathogens.

Therefore, studies of reliable, simple and time saving identification methods are required for both basic research on lactic acid bacteria and their applications in industrial food fermentations. These methods aim to distinguish genera and species.

Identification methods of lactic acid bacteria are conventionally based on morphological and biochemical criteria. Recently, they have highly developed by using DNA based analysis, and particularly by the Polymerase Chain Reaction (PCR) (Saïki *et al.* 1986).

A recent innovation is amplification fragment length polymorphism analysis by arbitrary primers (Welsh and Mac Clelland 1990). This strategy called **RAPD** (Random Amplified Polymorphic DNA) involves the enzymatic amplification of template DNA directed by one or more arbitrary oligonucleotide primers to produce a characteristic spectrum of products, a proportion of which can be polymorphic.

The procedure is fast, independent of prior genetic and biochemical knowledge of the organism tested.

The present work has been carried out as a contribution to this new method in the field of lactic acid bacteria identification. We describe the use of RAPD as an effective and rapid method to discriminate 12 *Lactobacillus* strains that were previously characterized in our laboratory by gas chromatographic analysis of cellular fatty acids (HRGC) (Wauthoz 1992).

Lactobacillus brevis NCIB 4617, NCIB 4036; *Lactobacillus buchneri* NCIB 8007, ENSI 9; *Lactobacillus casei casei* ENSI; *Lactobacillus confusus* NCIB 4037; *Lactobacillus fermentum* DSM 20052, CIP 53163, EPI 7, EPI 11; *Lactobacillus plantarum* DSM 20174, CIP A169.

Lactobacillus strains were grown under standardized conditions in MRS medium (Man, Rogosa and Sharpe) and total genomic DNA for RAPD was extracted from 3 ml of overnight culture following Cancilla (1992). RAPD was performed by the method of Mazurier (1992) using ten 10-mer primers (kit OPC-01, Operon) and the amplification products were separated according the size by agarose gel electrophoresis.

Using the oligonucleotide OPC-01 (TTCGAGCCAG) as primer, specific DNA fingerprints have been obtained for each of the studied strains (figure 1). The number of DNA bands in these fingerprints ranged from 1 to about 12 and differences in intensity were observed between these bands.

Although only a limited number of *Lactobacillus* strains were evaluated, these data demonstrate the ability of RAPD to differentiate between *Lactobacillus* strains that are not always discriminated by HRGC analysis and by conventional microbiological methods.

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LAST LECTURE

5 December 1995

TAXONOMY AND NOMENCLATURE OF THE FUNGI:
REASONS FOR A MYCOTHEQUE

RETROSPECTIVE[4]

G. L. HENNEBERT

Introduction

Philibert Biourge, in 1895, then assistant of Professor Jean-Baptiste Carnoy, studying the moulds of brewery malts, went to question the diversity of the *Penicillium* isolates, all being in greenish tinges close to that of *Penicillium glaucum*. Addressing the matter to Carnoy, he received this answer: "Then, Biourge, do a monographic study of the genus!" (Biourge in Glibert 1912, Hennebert 1979).

In 1956, Victor Estienne, my professor in plant pathology, observed with similar perplexity that "authors describe distinct species of *Botrytis* on the onion, *Allium cepa*" and asked me, "What are they, exactly? Are they not all *Botrytis cinerea*? You should study them." It was his suggestion for a PhD research, to solve a problem that he thought to be of host specificity: "Compare them through cross-inoculations in host plants." But cross-inoculations failed.

Professor N. Fabritius Buchwald (Fig.8), author of the *Studies of Sclerotiniaceae* (Buchwald 1949) in the steps of Prof. Whetzel of Cornell University, said to me in Copenhagen, "The matter is primarily taxonomical. There are many names of *Botrytis* species in the literature. Many are not *Botrytis*, some are, and among these, a number are host specific, others are not. Differentiation of the species is first of all morphological. You should compare fresh collections morphologically, from nature and in culture. You should look for the perfect stage in nature and find out the correct name to apply". His impulse, in 1957, launched me into the taxonomy and the nomenclature of the fungi.

The literature from the oldest to the most recent includes more than 380 species names in *Botrytis*, but many have become synonymous to names of taxa as distant as *Phytophthora*. The remaining names, often with old descriptions in Latin, too short or lacking illustration, were investigated through examination of the original or type material, when extant, from diverse herbaria.

In the meantime, I was exploring woods, swamps, ponds, fields, vineyards, orchards and vegetable plots, flower gardens, agricultural and

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[4] Last lecture delivered at UCL on December 5, 1995.

forest soils, even caves (Hennebert 1960b, 1971) collecting specimens, and isolated *Botrytis* strains in pure culture.

Most of the cultures were conidial and sclerotial. I was disturbed finding in some of them small globose conidia like *Penicillium* conidia, even in single *Botrytis* conidium culture. I could not obtain their germination and concluded that they were spermatia, first discovered by De Bary (1884) in *Botrytis* and described by Istvanffi (1905) and Brierley (1918) (Hennebert, 1960a).

The multiplicity of forms in *Botrytis* cultures, conidial, sclerotial, spermatial, expressed the pleomorphy found as a quite general character of the fungi (Tulasne 1851). Fungal pleomorphy is a phenomenon that was misunderstood and confused with polymorphism and 'transformism' by Carnoy (1870) who described the transformation of a *Mucor* into a yeast that turned to become a *Penicillium* (Hennebert 1979).

The forms observed in *Botrytis* were said to be asexual and "imperfect". Guided by the works of Whetzel (1945), Raabe (1938) and Buchwald (1949, 1953), who observed and described the sexual state of *Botrytis* species, I collected apothecia on sclerotia, together with conidiophores of *Botrytis*, in the field under *Allium ursinum*, *Ficaria verna* and *Caltha palustris*, representing three different species, *Botryotinia globosa* Buchwald, *B. ficariarum* Hennebert and *B. calthae* Hennebert and Elliott (Fig. 1-3) (Hennebert 1958, 1960a, Hennebert and Groves 1963). The apothecia, the sexual, ascosporic form of reproduction, were said to be "perfect" states of the asexual *Botrytis* states. Apothecia were also developing spontaneously in a pure culture of *Botrytis porri* Buchwald from *Allium porrum*, confirming its identity as *Botryotinia porri* (van Beyma) Whetzel (Fig. 4) (Hennebert 1960a, 1963b). The species is homothallic. But in heterothallic species to produce apothecia needs crossing.

To each state of the fungi, imperfect state and perfect state, a distinct Latin binomial name was given. This is heritage of the past when mycologists considered each reproductive form a distinct species. The most common species of the genus *Botrytis*, thus received the following names:

Sclerotium durum Pers.: Fr., described by Persoon (1794a) for the sclerotial form, belonging to the genus *Sclerotium* Tode: Fries (Tode 1790).

Botrytis cinerea Pers.: Fr., described by Persoon (1797) for the conidial form, renamed from the polynomial nomenclature of Micheli (1729).

Botryotinia fuckeliana (De Bary) Whetzel, described for the ascosporic sexual ("perfect") form of reproduction, the apothecium. The apothecial state was discovered on *Sclerotium echinatum* Fuckel, a synonym of *S. durum* Pers.: Fr., by De Bary (1866), who named it *Peziza fuckeliana* De Bary in honour of Fuckel, a name that Fuckel (1870) himself changed into *Sclerotinia fuckeliana* (De Bary) Fuckel. The genus *Sclerotinia* was divided by Whetzel (1945) on the basis of the organic connection to diverse asexual forms and *Botryotinia* connected to the form *Botrytis* as established by De Bary (1864, 1884, 1887).

The spermatial form of *Botrytis cinerea*, discovered in this species by De Bary (1884), belongs to *Myrioconium* Sydow (1912) but has not been named specifically. Spermatia, so-called by Tulasne (1856), are phialoconidia which rise from both external and internal phialides on hyphae.
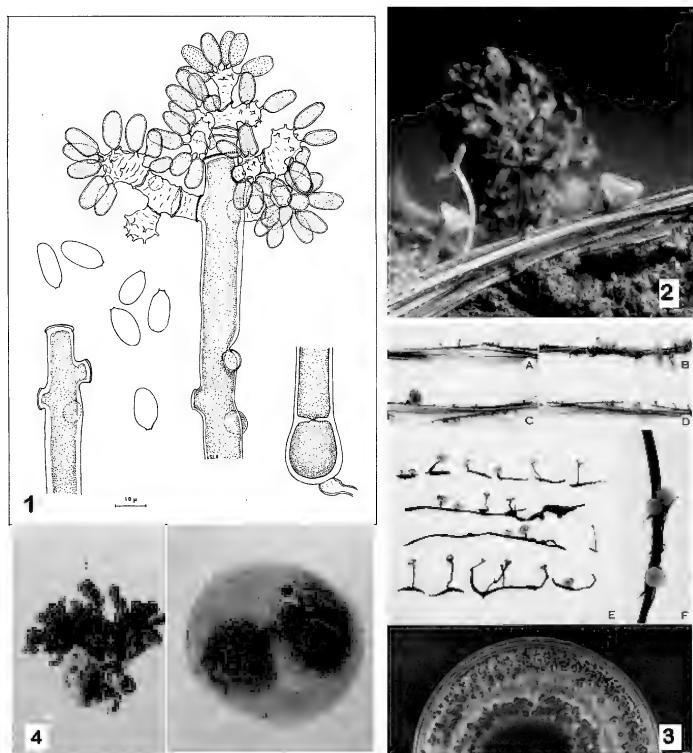
Fig. 1. *Botryotinia ficariarum* (*Botrytis ficariarum* anamorph) ; 2 and 3. *Botryotinia calthae*, apothecia and culture ; 4. *Botryotinia porri* (sclerotium and apothecium in culture.

Several names for one fungus species appeared contrary to Linné's principle and that of the International Code of Botanical Nomenclature, one taxon - one name. In such cases the Code prescribes the predominance of the name of the sexual form over the other names and tolerates however the use of the other names. That peculiarity of the nomenclature of the pleomorphic fungi struck me from the early days.

The embryo of a mycotheque

Soon I had hundreds of *Botrytis* isolates in pure culture beside isolates of many other fungi, an embryo of a fungus culture collection, a 'mycotheque' (Hennebert 1968d). "But why bring nature in small tubes?" questioned a relative of mine, long ago. Why a fungus culture collection? That is the question I wish to answer today, through an overview of 35 years of mycology.

Carnoy (1870) had already answered the question: "J'ai toujours cru que, pour faire sortir de l'enfance la science mycologique, il fallait cultiver les champignons, les cultiver toujours, les placer dans des milieux les plus divers, et, le microscope en main, suivre pas à pas leur développement. Lorsqu'on trouve une forme, au lieu de la décrire immédiatement comme une espèce nouvelle, n'est-il pas bien plus naturel de se demander d'abord si une culture méthodiquement variée la reproduirait toujours ou si elle ne pourrait pas en tirer autre chose? Aussi on doit savoir gré à tous ceux qui s'adonnent aux études expérimentales sur le développement et les métamorphoses de cette classe d'êtres singuliers." Here was an early promotion of the cultivation of the fungi in pure culture and of its use in experimental mycology to help fungal taxonomy.

Why a mycotheque? With Carnoy, I answer: to help elaborate a more natural taxonomy of the fungi, through demonstration and comparison in pure culture of their morphology and pleomorphy, and correlatively to resolve nomenclatural problems that pleomorphy may cause. Taxonomy and nomenclature were, to me, the prime reasons for building a mycotheque.

In those early days, I frequently visited the Centraalbureau voor Schimmelcultures, a well organized mycotheque, successively directed by Dr. Johanna Westerdijk, Dr A. van Beverwijk (Fig. 9) and Dr J.A. von Arx (Fig. 10) and where Prof. Biourge deposited *Penicillium* type strains in 1929.

The Mycotheque developed from that of Prof. Biourge at the University of Louvain, named MUCL, included 34,000 herbarium specimens and 24,000 living strains based upon them in 1994[5].

1. DEFINITIONS

Taxonomy and nomenclature are too often abhorred activities and thus eliminated from training in many universities. However, I wish to let them be seen more serenely.

Taxonomy and nomenclature are at the base of all activities of man who observes, thinks and communicates. Observation leads to thought, thought to concepts. Taxonomy is the result of observation and thought. Nomenclature results from thought and concepts. Taxonomy and nomenclature is at the base of all common knowledge.

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[5] MUCL preserves presently around 51.000 herbarium specimens and 35.000 living strains of fungi.

Observation of the diversity of things induces comparison and distinction, and leads to elaboration of concepts and to formation of words. If a concept is a category, the word is a noun; if it is an attribute, the word is an epithet. Categories are genera, epithets refer to species. That is taxonomy. Nouns are generic names, nouns plus epithets are species names. That is nomenclature. Common knowledge is made of taxonomy and nomenclature.

Fig. 5. Taxonomy and nomenclature in the old time (by an unknown mycologist).

The first approach of fungal biodiversity consists of taxonomy and nomenclature. The names of fungal species are, in the binomial nomenclature of Linné, a specific epithet combined with a generic name. For the stability of nomenclature, a reference specimen of the species, designated as type of its name and preserved under that name, guarantees the name application.

Classification is the direct product of taxonomy, by creation of categories of hierarchical rank: species, genera, families, orders, etc. When built on different criteria, classifications differ. Different classifications constitute different systems.

Identification is the recognition of an individual, a specimen, as belonging to a known species through its close similarity with the type or reference individual of the species, and application of the species name. If an individual does not belong to any described species, it represents a new species, perhaps a new genus, to be described, typified and named.

2. TAXONOMY

2A. Study of fungi starts in the field, by observation and collecting

The first step of any mycological study is the observation of the fungi in the field. To teach this basic requirement, Dr Stanley J. Hughes, the first day

after my arrival in Canada for a two-year postdoctoral stay (1960-1962), took me to the forest, in Gatineau Park, to collect microfungi, field shoes on feet, knife and basket in hands (Fig. 5).

Professor Richard P. Korf, who I visited at Cornell University in 1961 for the first time of many, was doing the same with his students: no mycology in class or laboratory before a discovery of the fungi in the field (Fig. 6). The bonnet of the car could serve as a bench to sort the collections. But take care: some fresh polypores, like *Ganoderma applanatum*, exude drops of so active enzymes that within one hour time you may have to let repaint the bonnet.

Nathalie Buffin, my assistant, repeatedly went collecting, whether thermophilic fungi in hot springs from coal tips in the Borinage, South of Belgium, or nematophagous *Arthrobotrys* species, or coprophilous or semi-aquatic hyphomycetes.

Mycology starts in, and proceeds from the field. It is necessary in the field to annotate the collected specimens with indication of the substrate, host, habitat, locality, collecting date, collector's name, and any other ecological information observable in the field. Necessary also is the microscope examination and the drawing of the fungus mounted when fresh, for, as I experienced, microscope slides are much better from fresh than from dried material. Drawings, made at the same magnification, and a written description on prepared format, help to attempt a first identification and apply a name, even a provisional one. Isolation of the fungus shall be made on different culture media, preferably by transfer of single spores, conidia or mycelial threads. The fungus is then labelled and registered with number and name. Finally, specimens are dried and preserved, together with microscope slides and dried cultures, in properly sealed and labelled envelopes in the herbarium (Hennebert 1968d).

2B. Building a mycotheque for experimental taxonomy

The *herbarium* is the first part of the mycotheque. Specimens are generally disposed by name (genus and species), either taxonomically (classes, orders, families) or alphabetically throughout. Both taxonomic and alphabetic disposition are subjected to name changes, but the former is also dependent on the classification. I adopted the alphabetical order. Packets are disposed like cards in boxes, what proved more flexible and space-saving than packets glued on herbarium sheets in folders, which I first used. Each set of specimens of the same species is separate and headed by a color card with the species name. Five suggestive colors were selected for species cards, grey for Myxomycetes, blue for Oomycetes, violet for Zygomycetes, red for Ascomycetes, brown for Basidiomycetes and green for Deuteromycetes, providing information on the class of the fungi.

Fig. .6. Dr Stanley Hughes on collecting fungi in the Gatineau Park,
near Ottawa, Canada, September 1960

Fig. 7 Dr Richard P. Korf at the end of a collecting trip with students
in a forest near Ithaka, N.Y., September 1961.

Fig. 8. Pr. Fabritius N. Buchwald, Copenhague, DK; 9. .Dr. Agatha van Beverwijk and 10. Pr. J.A. von Arx, successive directors of CBS, Baarn, NL (photo 9 of a drawing by Dr. G.A. de Vries).

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The *collection of living strains* is the second part of the mycotheque. Living cultures are disposed numerically and preserved in three different ways: on agar slants in two or more culture media under mineral oil, some also fresh and under water; lyophilized when sporulating; and cryopreserved at -140°C. Much research was carried out to select the optimal cooling speed and the cryoprotectant medium in lyophilisation and cryopreservation (Hennebert 1987a, 1987c, 1987d, Berny and Hennebert 1988, 1989a, 1989b, 1990a, 1991a).
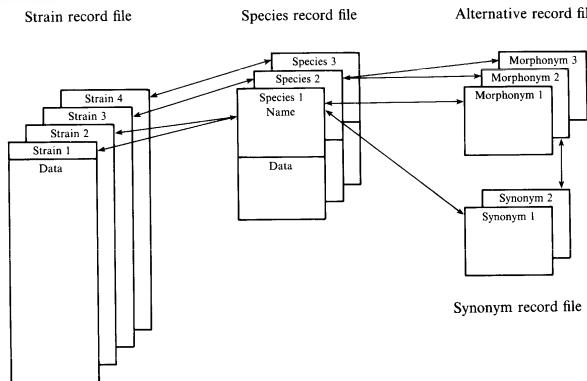
Fig. 11. The database is a set of four linked data sets, specific names and data, strain data, morphonyms and synonyms.

The *register* and the *database* make the third part of the mycotheque. All fungi collected are registered with a unique serial number. The number is applied to the specimen, the label, the slides, the drawing, the photographs, the descriptions, the living and dried cultures, the computed data set and the printed data sheet.

The numerical registration allows one to know the last number applied. Beside the numerical register, an alphabetical card system records the names and useful synonyms of the species with the numbers of the living strains represented in the collection. From 1985 onwards, computerization of the collection made the card system, not the handwritten register, obsolete. Strain and species data are recorded in the database, following a standardized scheme of data (Fig. 11) (Gams *et al.* 1988, Hennebert 1994a). The database allows recording and retrieval of the data, management and statistics of the mycotheque and the publication of the catalogues of strains (Hennebert *et al.* 1989, 1992).

The *iconotheque* is the fourth part of the mycotheque. It contains all drawings, descriptions and computerized datasheets of the collected fungi. The drawings are made at 1700x (Fig. 12), sometimes also 680 x or 170 x

magnification from natural material and, often only, from culture material. The iconotheque includes photographs as well.

An *alphabetical index of the fungal names* supported by a *mycological library* makes a fifth part of the mycotheque. This alphabetical index with bibliographic reference was gradually compiled from diverse indexes, from Streinz's *Nomenclator Fungorum* (1862), Saccardo's indexes, Petrak's indexes, to the most recent CAB *Index of fungi*. The compilation greatly helped bibliographical research.
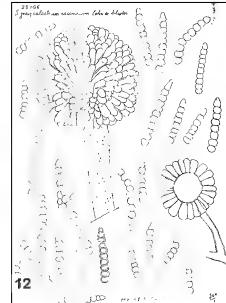
2C. Difficulty of interpreting old literature and tracing the type

Many names of fungi were created during the last two and half centuries. The interpretation of the ancient authors before 1900 and of their taxonomy remains uneasy. To interpret correctly old descriptions, it is necessary to
- understand Latin,
- be aware that fungi were described from nature, not in culture,
- take into account the tools of observation in current use at the time,
- know the precise meaning of descriptive terms and taxonomic criteria used at the time,
- know the value of measurement units of the period,
- remember that the rules of nomenclature applied were those of Linné until 1867, with some possible deviations peculiar to certain authors, and those of De Candolle and successors after 1867.

Tracing the type
In each case, tracing and carefully examining of the type, paratype or authentic material preserved in the author's herbarium or other herbaria is a requirement to make an enlightened interpretation of the description. Sometimes the type material is to be found, far away in a lost box above a herbarium cabinet, or covered with dust and mouse frass under the roof of a long deceased mycologist's house.

Necessity of type examination
Dr S. J. Hughes proposed to interpret adequately a two-line description like that of Wallroth's *Balanium* (Fig. 13) (Wallroth 1833). The type material of was necessary and made possible an adequate description in modern terms (Hughes and Hennebert 1961). At the same to justify the name *Oedemium didymum* (Schmidt) Hughes as correct for *Botrytis didyma* Schmidt 1817, required looking through one century of mycology and finding up to 5 synonymous genera and 16 synonymous species (Fig. 15, 16). Among the species
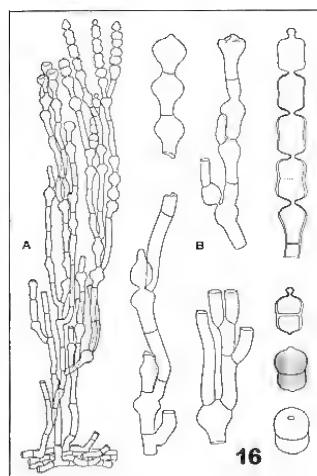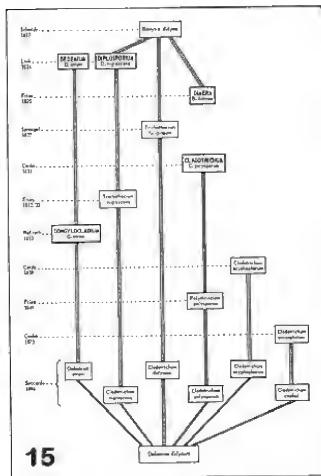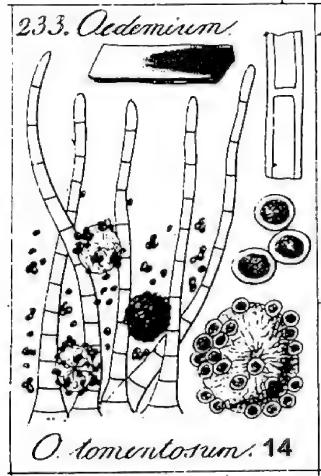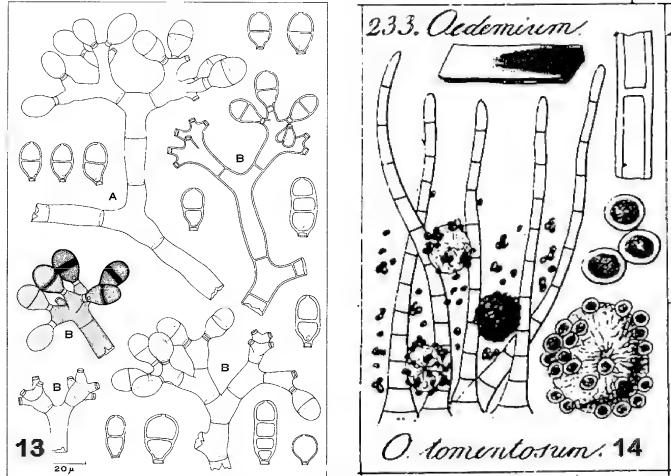
Fig. 13. *Balanium stygium* Wallroth; 14. *Oedemium didymum*, as *O. tomentosum* Corda after Corda 1837 and his type material; 15. Synonymy of *Oedemium. didymum*: five generic and seven specific names; 16. *Oedemium didymym* (Schm.)Hughes.

investigated, *Oedemium atrum* Corda was described and illustrated by Corda (1828) while he was only 19 years old. Corda's data could be interpreted only by examination of the type material, fortunately preserved in Prague. The specimen was composed of dematious sterile fungal setae mixed with globules that Corda (1833) recognized of heterogenous nature, being insect frass containing *Penicillium* conidia, three elements described by Corda as part of the fungus (Fig. 14). The decision to declare the name *Oedemium atrum* a *nomen confusum* could not have been undertaken without an examination of the type (Hughes and Hennebert 1963).

Iconotypes and neotypes

Dierckx (1901), student of Biourge, published in a short 6-page note, 22 new species of *Penicillium* that he had fully described and illustrated from pure culture, in a handwritten thesis. Unfortunately, when Dierckx went travelling in Indonesia, all his cultures were lost with the exception of *P. griseo-roseum*. On his return, he attempted to recover the species. Not very successful, he left notes and drawings to Biourge who succeeded to recover 20 of Dierckx 22 new species, and re-described them in his monograph of *Penicillium* (1923). In 1979, during an exhibition commemorating Biourge's microbiological carrier, a member of his family let us have a box from under the roof of Biourge's house containing the three plates of Dierckx's drawings (Fig. 17, 18, 19), but the handwritten thesis remains lost. The drawings are now considered as type material (iconotypes), which support Biourge's selected neotype cultures for Dierckx's species of *Penicillium* (Hennebert 1979, 1985).

Guidance of the protologue

If no original material can be traced, the originally published description, illustration and comments about habitat, locality etc., constituting the protologue, are the only data available to select a type. Any contemporary or later interpretation of the description of the taxon, and any application of its name by another author, should be regarded with caution.

Ostracoderma pulvinatum Fries, the only species of the genus *Ostracoderma* Fries 1825 (Syst. Orb. Veg. 1:150), was collected in Sweden and later recognized by Juel (1920). The fungus matched perfectly Fries's protologue of *O. pulvinatum* 1829 (Syst. mycol. 3: 214, hereunder) for which no type is available

1. *OSTRACODERMA pulvinatum.*

Sparsum aut gregarium, circumscriptum, rotundato - hemisphaericum, magnitudine varium, ¼ unc. circiter latum. Peridium crustosum, sed tenuissimum et fragillimum, laeve, glabrum, albidum, in medio collabens et evanescens. Sporidia minuta, globosa, argillacea, laxe coacervata, ipsi humo incumbere videntur. *In silvis, locis deustis, ad terram inter muscos, nobis aliquoties obvium. Ineunte autumno.* (v. v.)

Fig. 17. Orignal Dierckx' drawings of *Penicillium* species (1901): from left to right, 1st row, (first drawing lost), *P. candidofulvum* Dx., *P. aurantiobrunneum* Dx.; 2[d] row, *P. roseopurpureum* Dx., *P. citreoroseum* Dx., P. *citreonigrum* Dx.; 3[d] row, *P. corylophilum* Dx., *P. carneoviolaceum* Dx..
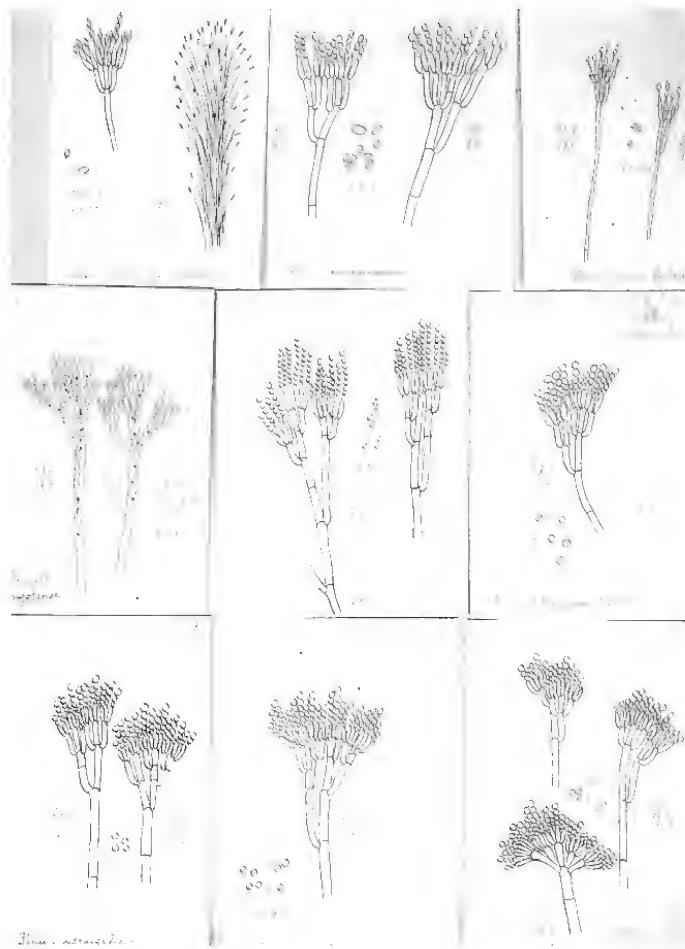
Fig. 18. Original Dierckx' drawings of *Penicillium* species (1901): from left to right, 1st row, *P. duclauxii* Delacroix?, *P. aeruginosum* Dx., *P. minioluteum* Dx.; 2^d row, *P. congolense* Dx., *P. elongatum* Dx., *P. glaucum* Link?; 3^d row, *P. atroviride* Dx., *P. verrucosum* Dx., *P. griseobrunneum* Dx..

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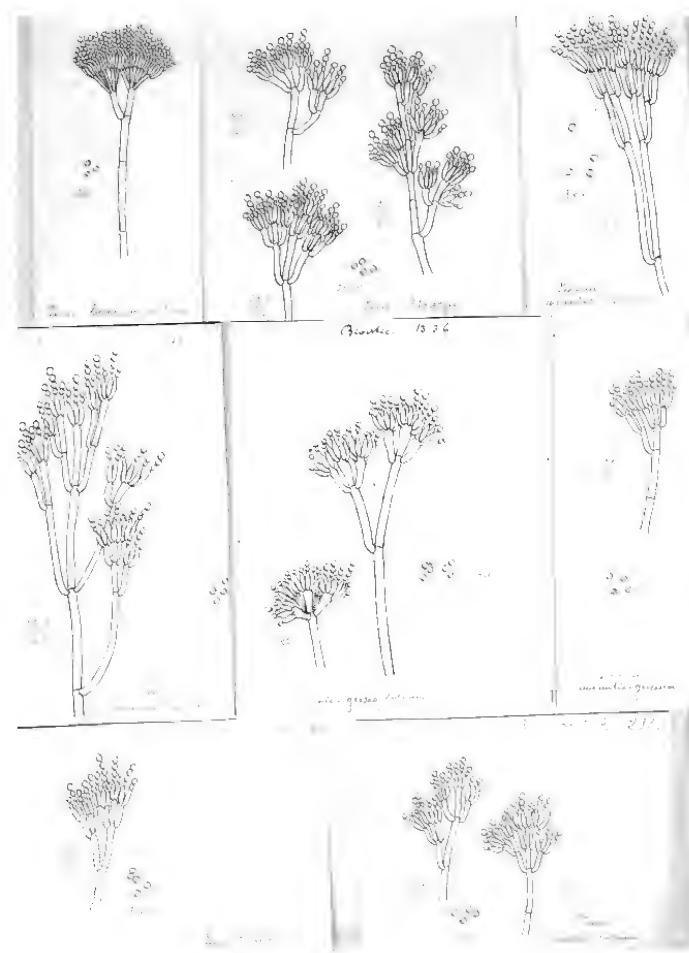
Fig. 19. Original Dierckx' drawings of *Penicillium* species (1901): from left to right, 1st row, *P. brevicompactum* Dx., *P. biourgei* Dx., *P. aurantiocandidum* Dx.; 2d row, *P. brunneorubrum* Dx., *P. griseofulvum* Dx., *P. aurantiogriseum* Dx.; 3d row, *P. hirsutum* Dx., *P. griseoroseum* Dx..

Juel considered the fungus conspecific of *Hyphelia terrestris* Fries 1849 and therefore synonymized *Ostracoderma* with *Hyphelia* Fries 1825. But *Hyphelia* Fr. is not available because of misapplication of his type species name (Hughes 1958). In another hand Hughes (1958) synonymized *Chromelosporium* Corda 1833 with *Ostracoderma* Fries 1829, but did not consider the absence of a "crustose peridium" in the former, clearly indicated in Fries's protologue of *Ostracoderma pulvinatum*, giving the fungus the appearance of a *Myxogaster* ("*Facile igitur crederes esse Myxogatrem*") and also observed by Juel. This feature forced the rejection of Hughes' synonymy, although conidiogenesis in both genera is similar, and the distinction of *Chromelosporium* and *Ostracoderma* (Hennebert 1973).

The type locality

In absence of any original, type or authentic material of a fungus, but guided by the published protologue, one last resource is to go collecting the fungus anew in the type locality. When the locality is not destroyed, the fungus can be found unless it is of rare occurrence. I discovered *Arachnophora fagicola* Hennebert (Fig. 26) from a few conidiophores only, just enough to

prepare a microscope slide, on a cupule under a beach tree in Parc d'Arenberg, Heverlee, Belgium (Fig. 20). Later wanting to provide sufficient type material for herbarium deposit in view of publication, I had to examine eight thousand beach cupules from the same locality to finally have eighteen minute colonies of that new fungus to serve as a type (Hennebert 1963a).

Tracing the type host

The same conduct is to be followed when a living culture is wanted for reconsideration of a rare species. *Seaverinia geranii* (Seaver and Horne) Whetzel (1945) had been found on *Geranium maculatum* in localities of New York and Wisconsin States five times in 50 years, successively by Seaver, Davis, Whetzel, and Korf. Ten years after the last collection by Korf, in the

forest along the Cayuga Lake near Ithaca, I went collecting on the same spot and found the species (Fig. 21). Fresh single ascospores could cultivated and yielded the verrucose conidial state *Botrytis geranii* Seaver. After a proposal by Buchwald (1949), I redisposed the conidial state in the new form-genus, *Verrucobotrys* Hennebert, as

Verrucobotrys geranii (Hennebert 1973).

Ex-type cultures

Sometimes, natural type or authentic material is lost, either not deposited in herbarium, or destroyed, but *ex-type* cultures are preserved in culture collections. Should a taxonomist describes or re-describes a fungus from culture, he should then deposit it in a dried state in herbarium. So did I with *Wardomyces columbinus* (Fig. 37) and *Asteromyces cruciatus* (Fig. 27) (Hennebert 1962), of *Custingophora olivacea* (Fig. 28) (Stolk and Hennebert 1968), of *Lomentospora prolificans* (Fig. 40) (Hennebert and Desai 1974), and many others isolated from soil (Hennebert 1968a, 1968c, 1969). Conversely, in order to spare type material in herbarium, it is recommended to deposit living ex-type isolates in culture collections (Banno *et al.* 1993, 1994).

Tracing earlier names

Many taxonomists dare to describe and publish rapidly a species they consider as new, taking the risk to see it synonymized soon after. To be certain that the species has not yet been described or the name already used, before publishing a new species or a new name, a full survey of mycological literature and indexes is necessary.

A *Wardomyces*-like fungus isolated from soil in Belgium and considered a new species was also isolated from soil in the Netherlands. After screening the available literature for some times, I prepared a description of the new species for publication, when I received a reprint from Subramanian describing the fungus as *Adoghamina ruchira* Subramanian n. gen. n. sp. Two weeks later I found Barron's description of the same fungus as *Gilmaniella humicola* Barron n. gen. n. sp., preceding by a few weeks the publication by Subramanian. The synonymy remained to be published (Hennebert 1968b).

Nathalie Buffin collected an interesting white arbuscular hyphomycete bearing cylindrical conidia and forming white patches on semi-immersed dead leaves at a pond in the Lauzelle Forest, Louvain-la-Neuve (Fig. 23). We though the fungus to be undescribed. We composed a descriptive name, *Cylindrodendrum album*. Checking for a possible earlier use of the name in several indexes, we found that the name had been used
already by Bonorden (1851) (Fig. 22) which description and illustration were matching our fungus exactly. There is no type material left of Bonorden's species, but a search in other herbaria revealed that the fungus had been collected twice in 120 years after Bonorden. Our collection was désignated neotype (Buffin and Hennebert 1984).
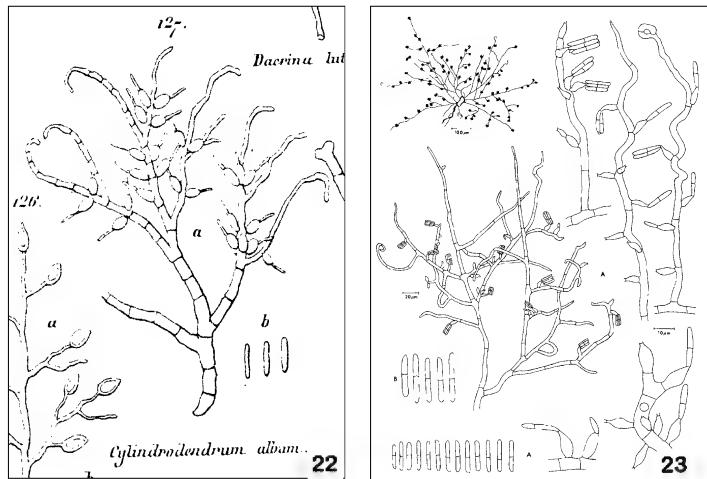
Fig. 22. *Cylindrodendrum album* Bonord. original drawing; 23. Drawing made by N. Buffin from fresh collection and culture.

Publishing rapidly

Even after searching for guarantee of the novelty, it is important to publish rapidly. As very few journals were editing manuscripts with a delay shorter than 6 months, Richard P. Korf and I decided to edit a new journal offering only a short delay together with quality, designed to promote the taxonomy of fungi and lichens (Hennebert and Korf 1974). Created in July 1974, *Mycotaxon* has published, in 1995, 67 volumes of around 540 pages each, about 36000 pages in 24 years[6].

2D. Evolution of taxonomic criteria from morphology to morphogenesis

Taxonomy of fungi evolved greatly after Linné with development of tools for a finer observation and characterization of life. New characteristics of fungi are tested as taxonomic criteria. That evolution requires constant adaptation by the taxonomist.

It does not mean that traditional criteria such as static descriptive morphology are abandoned. To the contrary, they make the basis of present taxonomy, but enriched by morphometry, morphogenesis, sporogenesis, anatomy, physiology, biochemistry and genetics.

It is possible to distinguish four periods in the evolution of taxonomical criteria: a first period of static morphology from field material, a second

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[6]Mycotaxon published its 100th volumes in 2007.

period enriched by morphogenesis in experimental mycology (pleomorphism, sexuality, culturing), a third period aided by physiology and biochemistry, and a fourth, the current period, revised by genetics and phylogeny.

From Linné (1753), through Tode, Persoon, Link, Fries, Corda, Wallroth, Fresenius, Fuckel, De Bary, R. & F. Tulasne, Saccardo and many others until the 1850-1880s, taxonomic criteria in fungi were merely morphological and static, based on the observation of natural material and evolved from macromorphology to micromorphology with the development of the microscope.

Then morphogenetic criteria were added owing repeated developmental observations in nature and lately helped by use of aseptic culture on liquid substrates by Pasteur (1866) and on solid substrates by Brefeld (1875, 1881)

Presumed sexually functioning organs in fungi were observed by Ehrenberg (1820) in Zygomycetes, by Léveillé (1837) who described the hymenium of Basidiomycetes, by Tulasne (1856), who first described "spermatia", and De Bary (1863) who observed the "carpogone" and the "pollinode" in the Ascomycetes. Brefeld (1872) observed sexual fusion in Zygomycetes and other fungi. Stahl (1877) was the first to follow the fusion of spermatia with the ascogonial hypha he called "trichogyne" in Ascolichens. Blakeslee (1904) made the fusion of heterothallic partners in Zygomycetes. These discoveries led to the differentiation of sexual sporogenesis from conidiogenesis.

Fresenius (1850) already described the conidiogenesis of *Botrytis cinerea* (Fig. 24). The development of several kinds of secondary spores, or conidia, in addition to spores suspected to be sexual, led L. Tulasne (1851) to define pleomorphism in fungi. Numerous examples of this pleomorphism were illustrated in the Ascomycetes by L. Tulasne and C. Tulasne in the *Selecta Fungorum Carpologia* (1861-1865) and in the Basidiomycetes by De Seynes (1874) and in all groups of fungi by Brefeld (1872-1912).

Availability of the fungi in living culture greatly favoured the observation of pleomorphism and conidiogenesis and its use in taxonomy. An early example of the advantage of cultures was the morpohogenetic study of several *Penicillium* species made in vitro by Brefeld (1874).

More recent mycologists, Vuillemin (1910), Mason (1933), Hughes (1953) and Tubaki (1958), defined and described different types of conidiogenesis,
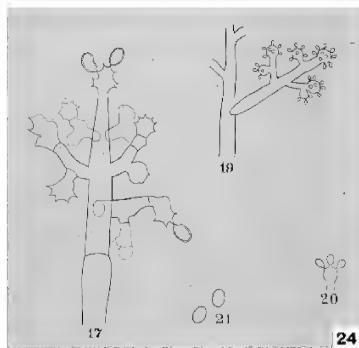
and used them as criteria for generic distinction of asexual fungi. Most of them were confirmed by electron microscopy (Samson and Cole 1970).

2E. Development of morphogenetic criteria: the unitary parameters of conidiogenesis

The conidiogenetic system of Hughes (1953) and Tubaki (1958) guided my descriptions and illustrations of the conidial fungi. On basis of conidiogenesis, were excluded from the genus *Botrytis*, species with blastic synchronous conidia but dissimilar from what was called the *Botrytis cinerea*-type (Whetzel 1945), belonging to *Chromelosporium, Haplotrichum, Mycotypha, Nematogonium and Ostracoderma*, species with sympodial blastic conidia belonging to *Beauveria, Calcarisporium, Cephalotrichum, Conoplea, Costantinella, Hansfordia, Nodulisporium, Rhinocladiella, Rhinotrichum* and *Virgaria*, species with phialidic conidia referable to *Verticillium*, species with retrogressive conidia as in *Cladobotryum* and species with arthric conidia which were disposed in *Geomyces* (Hennebert 1960a).

At the 1st Kananaskis conference on the *Taxonomy of the Fungi Imperfecti* (Kendrick 1971), advances were made in the understanding of conidiogenesis. Several new terms proved to be useful: among them, "holoblastic" and "holoarthric", "enteroblastic" and "enteroarthric", that I proposed to characterize the ontogeny of the conidia in relation to the wall of the conidiogenous cell. Also emphasis was given to the ontogeny of the conidiophore itself, beside that of conidia (Pirozynski 1971). Considering the conidiophore also, I segregated several form-genera among the *Botrytis*-like species (Fig. 25). A first group of them is linked, or presumably so, to operculate Discomycetes, order Pezizales, distinct from another group linked to the inoperculate Discomycetes, order Helotiales, family Sclerotiniaceae. In Pezizales, the new form-genus *Dichobotrys* Hennebert (Fig. 25-5) was described for the conidial form of *Lamprospora, Sphaerosporella*, and *Trichophaea* species, while two species of form-genus *Chromelosporium* Corda are conidial states of *Peziza* species. *Botrytis*-like form-genera presumably related to operculate discomycetes, *Pulchromyces* Hennebert (Fig. 25-7) and *Phymatotrichopsis* Hennebert (Fig. 25-8) were described as new, beside the "peridial hyphomycetes" *Ostracoderma* Fries and *Glischroderma* Fuckel (Fig. 25-10) (Hennebert 1973, Hennebert and Korf 1975, Bellemère and Hennebert 1979, Sutton and Hennebert 1994, Korf 1994).

In Sclerotiniaceae, distinctive features of conidiomata were enhanced as generic criteria to discriminate, beside *Botrytis sensu stricto*, three distinct new form-genera, *Streptobotrys* Hennebert (Fig. 25-2) for the twisted conidioma of *Streptotinia*, *Amphobotrys* Hennebert (Fig. 25-3) for the dichotomous conidioma of one *Botryotinia* species and *Verrucobotrys* Hennebert (Fig. 25-4) for its verrucose conidioma of *Seaverinia* (Hennebert 1973).
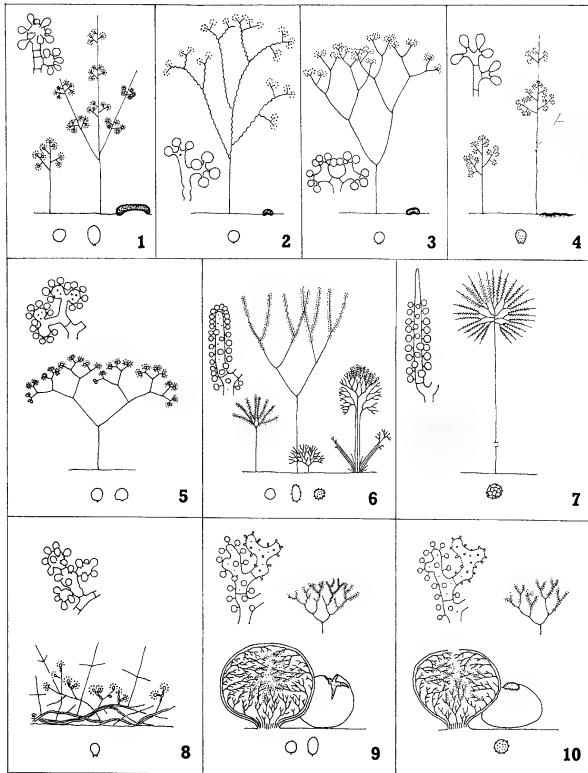
Fig. 25. Diagrammatic illustrations of coniophomata of 1. *Botrytis*; 2. *Streptobotrys*; 3. *Amphobotrys*; 4. *Verrucobotrys*; 5. *Dichobotrys*; 6. *Chromelosporium*; 7. *Pulchromyces*; 8. *Phymatotrichopsis*; 9. *Ostracoderma*; 10. *Glischroderma*.

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However, the more conidial fungi were examined, the more intermediate categories of conidiogenesis were discovered: *Wardomyces* and *Asteromyces* species have solitary blastic or thalloblastic conidia born in basipetal succession on the conidiophore (Hennebert 1962), *Arachnophora fagicola* (Fig. 26) has solitary blastic conidia borne successively trough percurrent proliferation of the conidiophore (Hennebert 1963a), *Sporoschismopsis moravica* (Fig. 29) produces successive arthric conidia borne in percurrently successive phialides (Holubova-Jechova and Hennebert 1972).

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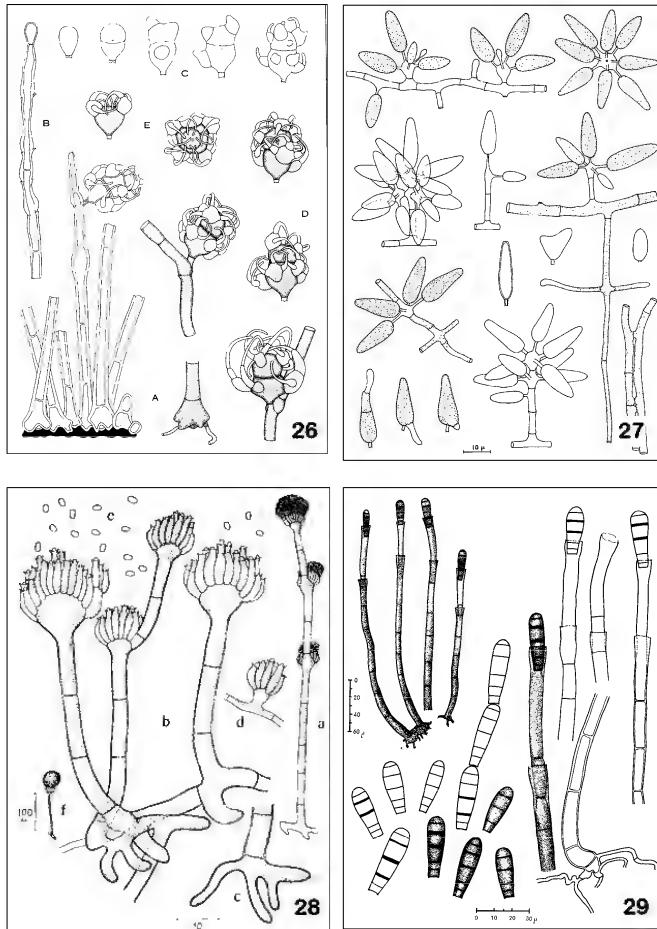
Fig. 26. *Arachnophora fagicola* Hennebert; 27. *Asteromyces cruciatus* F.–Me. Moreau ex Hennebert 28. ;*Custingophora olivacea* Stolk, Hennebert & Klopotek; 29. *Sporoschismopsis moravica* Hol.-Jech. & Hennebert

I thus progressively developed a more detailed, yet still unsatisfactory, scheme of conidiogenesis for my students (Hennebert 1982). Adressing the need for a finer analysis of conidiogenesis, I introduced the concept of *unitary parameters* acting together or successively in each specific conidiogenesis, instead of describing categories or types of conidiogenesis (Fig.30).
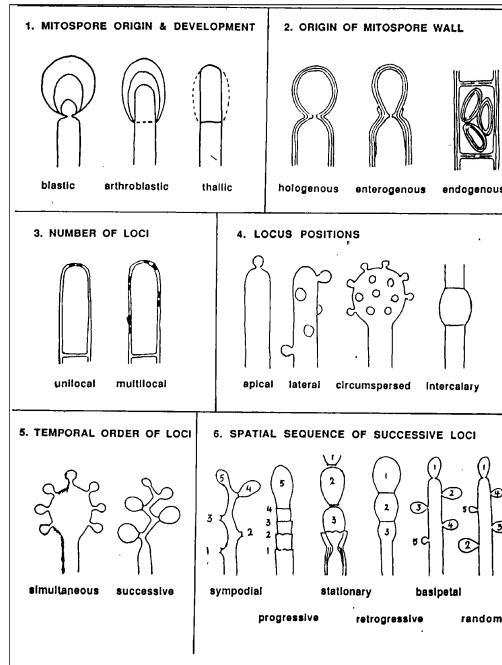
Fig. 30. One of the plates figuring some of the parameters, the combination of whichh shall define a conidiogenic pattern (Hennebert in Hawksworth 1994b)

The parameters are numerous and should be defined under a univocal descriptive terminology. A first and tentative analysis of a non-exhaustive number of unitary parameters of conidiogeneis was presented at the Ascomycetes Conference in Paris in 1993 (Hawksworth 1994b). When eventually fully refined, the unitary parameters should allow the characterization of any conidial fungus and hopefully, when computerized, serve as a basis for an expert identification system of conidial fungi (Hennebert and Sutton 1994).

2F. Value of cultures in demonstrating pleomorphism and variations in fungi

Like Biourge in 1892-93 who spent a year in both Pasteur and Hansen's laboratories learning the single cell culture (Hanssen 1886), I spent two years

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in Ottawa where Dr J. W. Groves taught me the single ascospore culture and the spermatization technique.

Single spore culture has been a great help in the separation of close species. I used single spore culture currently in *Penicillium*, and other genera for isolation of strains. Mass conidial colonies often sectorize, showing variants. Strains received for the collection, particularly ex-type strains, were regularly checked for homogeneity from 50 single spore cultures. In an attempt to rebuild the Biourge's *Penicillium* collection, type strains returned from other collections were found to contain up to 4 variants. Indeed, mutations occur during maintenance and preservation of ex-type cultures and selection of the original strain has frequently been necessary. In an experiment eight different variants were isolated from a homogeneous parental strain of *Penicillium expansum* following exposure to diverse preservation processes (Fig. 31) (Berny and Hennebert 1990a).
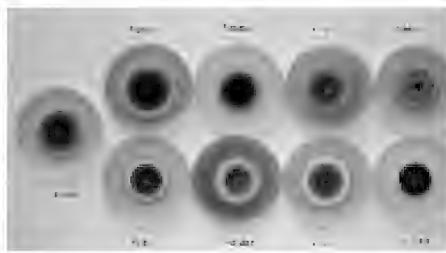
Fig. 31. Eight variants of *Penicillium expansum* wild strain (left) obtained from monospores after preservation processes.

Cultures help to demonstrate pleomorphism, as well as variability and mutability. Culture under diverse conditions allows the fungus to reveal itself in more facets than expected. An example is the dimorphism of many yeast and yeast-like fungi, even *Saccharomyces cerevisiae*, which turns filamentous under nitrogen starvation. *Moniliella pollinis* (Hennebert and Verachtert) De Hoog turned from arthric conidia to a sympodial budding yeast depending on the shaking speed in liquid culture (Dooms *et al.* 1971). Only through single spore culture, it could be demonstrated that spermatia belong to *Botrytis cinerea.*

Analysis of conidiogenesis In culture helped to reduce the taxonomy of the genus *Botrytis* to some thirty six species (Hennebert 1958, 1960a, 1963b, 1964a, 1966, 1969, 1973). Cultures also helped to demonstrate the sexual-asexual connection. Through crossing single conidial isolates of *Botrytis* by spermatization of sclerotia, apothecia of heterothallic *Botryotinia ficariarum* and *Botryotinia ranunculi* could be obtained in pure culture (Hennebert and Groves 1963). Homothallic species like *Botryotinia porri* can fruit easily. Similar proof of pleomorphic connection In yeasts were also atteined through rDNA sequencing from cultures.

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2 G. Classification of asexual fungi with sexual fungi

With or without cultures, another way to infer taxonomic relationship of conidial fungi in absence of a sexual reproduction, is by analogy of their morphology. In *Chromelosporium* three species only, *C. fulvum* (Link: Fr.) McGinty *et al.*[*C. ollare* (Pers.) Hennebert] (Fig. 32), *C. macrospermum* Hennebert and *C. trachycarpum* Hennebert, are conidial states of *Peziza* species, but the type species *C. ochraceum* Corda and six other species are only presumed to be states of Operculate Discomycetes by analogy, although they had often been considered states of Basidiomycetes such as *Hypochnus* or *Tomentella*. At the same *Phymatotrichopsis omnivora* (Duggar) Hennebert is considered by some authors a state of *Hydnum* or *Trechispora*.
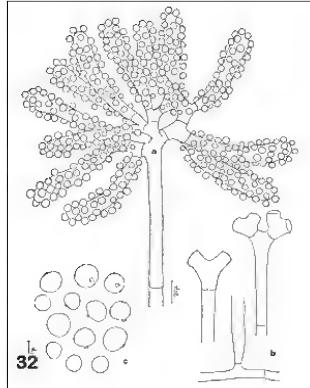
Ostracoderma Fr. and *Glischroderma* Fuck. are similarly grouped with conidial states of Operculate Discomycetes. A peridium, absent in the others, gives to *Ostracoderma pulvinatum* and *Glischroderma cinctum* Fuck. the external pulvinate smooth appearance of the myxomycete *Lycogala*. A closely related peridial fungus, *Glischroderma cinctum* Fuckel was described by Fuckel (1870) in the Gasteromycetes, and distinguished from *Ostracoderma*. Another 'peridial hyphomycete' with elliptical conidia, found by Bresadola, was named *Lycogala torrendii* as if a Myxomycete. It was renamed by Torrend (1913) *Lycoperdellon torrendii* and disposed in the Gasteromycetes. Heim and Malençon (1933), Heim (1934, 1949), Dissing and Lange (1962), Malençon (1960, 1964), Demoulin (1966) placed both genera in the Gasteromycetes and families were erected for them. Zeller (1948) rightly placed the fungi in the Deuteromycetes. Because of the analogy of the conidiogenesis with *Chromelosporium* states of *Peziza* species, following Donk (1962), I chose to dispose *Lycoperdellon* as synonym of *Ostracoderma* Fr. and with *Glischroderma* among conidial states of Operculate Discomycetes. *Glischroderma cinctum* and another undescribed species of *Glischroderma* Fuckel, with a glutinous peridium, were found by Korf and his students in the surroundings of Ithaca, NY. (Korf 1994).

The presumed position of *Ostracoderma* (= *Lycoperdellon*) and *Glischroderma* amongst conidial forms of certified Operculate Discomycetes (Hennebert 1973) was strongly supported by the presence of ascomycetous septal pores demonstrated in SEM by Bronchart and Demoulin (1975).

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Ultrastructure is indeed another way approach relationships between conidial and sexual fungi and their phylogeny.

2H. Relative aid of additional criteria

Physiological characters

After the promotion of physiological characteristics in yeast taxonomy by Guilliermond (1912), some additional characteristics, enzymatic activities, requirements in growth factors and tolerance to environmental conditions, were added. Those criteria are still in use (Berny and Hennebert, 1990b). In 1985, I initiated the miniaturization of all physiological tests of assimilation, fermentation, enzymatic activity, requirements and tolerances, using 96 well microplates read by optical densitometry. The method has greatly increased test rapidity. It was presented at the 8th International Symposium on Yeasts in Atlanta in 1992. Today, the microplate results, together with yeast morphological and genetic data, are integrated in the computer-assisted expert identification system BCCM/ALLEV-2 created by Vincent Robert at MUCL (Robert *et al.* 1994, 1997) and presented again as a poster at the MUCL centenary symposium (Evrard and Hennebert 1994)[7].

Biochemical characters

The development of analytical methods enhanced extensive biochemical investigations on both filamentous and yeast-like fungi. However the new criteria are so far applicable to sufficiently large fresh biomass of fungi from culture, or nature. Their application to type material in herbaria is excluded. That is a handicap of the biochemical approach.

Dominant Q co-enzymes have become a good generic criterium and a good indicator of the generic homogeneity. Applied to *Candida* the genus was found heterogeneous and composed of four distinct groups (Vancanneyt *et al.* 1991b, 1994a).

Enzyme and protein profiles have provided taxonomic help at the species level and lower ranks (De Bertoldi and Hennebert 1977, Berny and Hennebert 1991b, Hennebert 1996b, Hennebert and Vancanneyt 1998). Whole cell protein profiles proved to be useful in confirming by maximum similarity the organic connection established otherwise between asexual and sexual strains in both ascomycete and basidiomycete yeasts. The same degree of similarity between unconnected anamorph and teleomorph species led to presume of their close relationship, if not their ontogenic identity (Vancanneyt *et al.* 1990, 1991a, b, c, 1992, 1994b).

Genomic characters

Genomic analysis for fungal taxonomy developed rapidly. Polymorphism of fragmented DNA (RFLP) demonstrated the relation of

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[7] The System ALLEV later received further developments and world wide distribution as BIOLOMIX by the CBS, the Netherlands.

produced mutants with the parent strain of *Penicillium expansum* (Cercel *et al.*, 1998a, 1998b). Ratios in guanidine and cytosine confirmed similarities observed in protein profiles of yeasts (Vancanneyt *et al.* 1991c, 1994a).

Here also, genomic analysis is up to now applied only to living strains in culture, less easily to dried type material or small nature collection. The strains currently analysed are otherwise classically pre-identified. Taxonomic conclusions inferred from genomic analysis might therefore depend on the identifications of reference sequences. Since all names are obligatory linked to type material preserved in a herbarium, it is highly recommended that molecular taxonomists analyse type specimens and authenticated ex-type strains. Neglecting this basic requirement would be equivalent to ignoring 240 years of taxonomy and nomenclature (Gouliamova and Hennebert 1994a, 1994b, 1995)).

With that in mind, sequencing of nuclear rDNA or RNA associated with numerical analysis applied on ex-type strains and other reference strains provided much valuable data for both taxonomy and phylogeny of ascomycete and basidiomycete yeasts (Hendricks *et al.* 1989, 1991, 1992, Van de Peer *et al.* 1992, Wilmotte *et al.* 1993, Gouliamova and Hennebert 1994a, 1994b). They allowed confirmation of the anamorph-teleomorph connection in all cases tested and detection of presumed such connections in some other cases. Sequencing part of 18S RNA and the region ITS1-5.8S rDNA-ITS2 of ex-type or ex-neotype yeast strains, *Saccharomyces cerevisiae* and some reputedly synonymous species were compared.. *S. chevalieri* and *S. ellipsoideus* were found synonyms of *S. cerevisiae*, while *S. pastorianus*, *S. paradoxus*, *S. uvarum* and *S. bayanus* were confirmed to be distinct species (Gouliamova and Hennebert 1994b, 1998). Using the same ITS region, specific distinctions and seveal new species were established among thirteen unidentified strains of *Lipomyces,* otherwize detected by DNA reassociation (Gouliamova and Hennebert 1995).

3. NOMENCLATURE

Nomenclature Is linked intimately to taxonomy, as language is linked to concepts, and as locality names are to each destination box at the post office.

3A. Typification: the basis of nomenclature

Typification of names is one of the two pillars of botanical nomenclature stability, the other being priority of names.

Automatic typification of a new name by the type of an earlier name included in the protologue was the rule until the Montreal International Code of Botanical Nomenclature (Lanjouw 1961).

Interpretation of cases differs whether names are applied to the same taxon, or to two different taxa, one designated by the earlier name, the other described under the new name.

When a recent name is published for a taxon while an earlier legitimate and priorable name for the same taxon is cited as a synonym in the protologue and should have been used, automatic typification applies and the recent name is nomenclaturally superfluous and illegitimate. It was the rule until 1966. If the author in some way excludes the type of the cited earlier name, e.g., by designating a type of the new name for the *same* taxon, the new name with its type should be accepted and automatic typification should not apply. The result would be that the new name is legitimate and taxonomic synonym of the earlier name. That was the proposal made by Weresub and Hennebert (1963), accepted by the Edinburgh Code (Lanjouw 1966).

But often authors misinterpreted the description of an earlier species and therefore misapplied its name to a *different* taxon. There is then a conflict between the taxon represented by the earlier name cited as a synonym and its type and the taxon as described. The question in interpreting such mycological literature was: is the intention of the later author to be respected or automatic typification applied?

In such cases of misapplication of an earlier legitimate name, the Edinburgh Code (1966) maintained the automatic typification to be applied "*in all circumstances*" (Art. 7), no regards to the intention of the later author. The new name is then obligate synonym of the earlier one. That is the case of *Hyphelia* Fr.

Persoon (1794b) had described *Trichoderma roseum* and deposited a specimen on *Populus* bark in his herbarium in Leiden. Hoffmann (1795) collected the same species, named it *Trichoderma rosea* Pers. and described the conidia under the lens as oblong, phaseoliform and uniseptate. Later Persoon (1801) listed his *Trichoderma roseum* Pers. with reference to Persoon 1794 and Hoffmann 1795, adding its occurrence on *Salix* bark based on another specimen in his herbarium. Link (1809) erected the new genus *Trichothecium* Link with uniseptate conidia on the basis of *Trichoderma roseum* Pers.

Fries (1825) erected the new genus *Hyphelia* Fr., and included in the same description the name of one species "*Hyphelia rosea*", based on *Trichoderma roseum* Pers. which he explicitly propoposes as type, but commenting "not *Trichothecium* Link". In the same work, Fries recognized the genus *Trichothecium* Link, with the comment that it differs from *Trichoderma roseum* Pers.

. Fries (1829) in the *Systema mycologicum*, already written at the time of the publication of the new genus *Hyphelia* in 1825, indicated having seen a Persoon specimen with reference to Persoon 1801. Hughes (1958) indeed examined Persoon's three authentic specimens of *Trichoderma roseum* Pers. in Leiden herbarium and recognized *Trichothecium roseum* (Pers.) Link: Fr Fries might have had in hand a poor specimen from Persoon like the one deposited in 1801 and misinterpreted Persoon's description, consequently misapplying the name. *Hyphelia* Fr., based on a misapplication of the type

species name *Trichoderma rosea* Pers. is therefore obligate synonym of *Trichothecium* Link and therefore not available for the species Fries called *Hyphelia terrestris* Fr. (*Chromelosporium tuberculatum* (Pers.) Hennebert) (Hennebert 1973)

3B. Pleomorphy in fungi and nomenclature

Linné decided his taxonomy to be based on sexual reproductive structures. In the introduction of the *Philosophia botanica* (1753) he classified plant taxonomists according to their basic principles of classification and qualified himself as "sexualist" (Fig. 33). Consequently, fungal sexuality and pleomorphism being unknown at the time, any distinct form of reproduction in fungi was considered a distinct species and named.
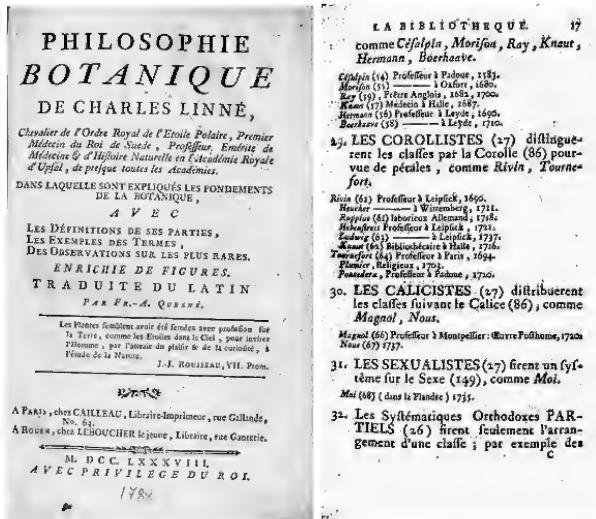
Fig. 33. Linné (1753) declares himself a "sexualist" taxonomist.

Corda (1839) described *Echinobotryum parasitans* as a parasite of *Stysanus caput-medusae* ignoring that it was another form of spores of the same fungus (Fig. 35). Even Saccardo (1882) and Penzig (1882) made the same 'mistake' when accepting *Echinobotryum citri* as a parasite of *Stysanus monilioides* , later corrected as *Echinobotryum atrum*(Fig. 36).

Tulasne (1851), who called *pleomorphy* the coexistence of several forms of reproduction in fungi, demonstrated the organic connection between ascosporic fungi and conidial fungi as the "perfect state" (which they presumed to be sexual) and the "imperfect state or states" of the same

species, as several conidial forms of reproduction may exist in the same perfect species (Fig. 34). Number of pleomorphic fungi had therefore more than one name. Tulasne (1851), in agreement with Linne's principle, asserted the predominance of the name of the perfect sexual state upon the names of the imperfect states, to designate the whole fungus (Hennebert 1971).
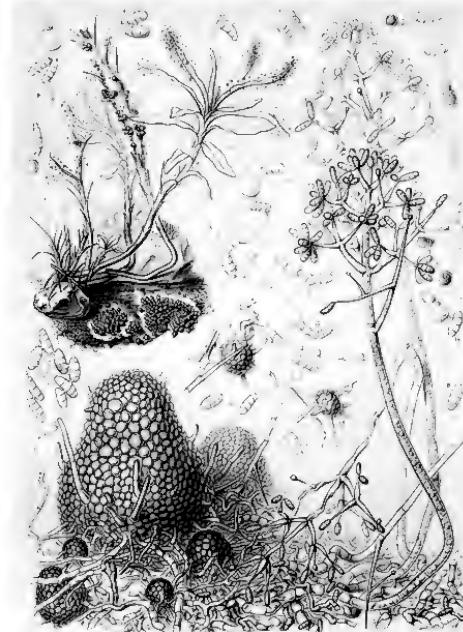
Fig. 34. Pleomorphy: *Hypomyces rosellus* and anamorph *Cladobotryum dendroides* (L.R. & C. Tulasne, III, 1865)

However, vast amount of conidial species remained, and still remain, without connection to a sexual form. Consequently, Fuckel (1870) classified the fungi in two major groups, the *Fungi Perfecti* and the *Fungi Imperfecti*. Saccardo (1889) named them *Fungi Superiores* and *Fungi Inferiores*, the latter being "metagenetic forms" (*formae metageneticae,*) which he designated later *Deuteromycetae* or *Fungi secundarii* (Saccardo 1899).

Although the *Lois de la Nomenclature Botanique* Art. 15 by De Candolle (1867) reinforced the principle "one taxon - one name", Saccardo (1904) in his *De Diagnostica et nomenclatura mycologica*, recommended to classify duly demonstrated pleomorphic fungi by their appropriate names in *Fungi Perfecti* and *Fungi Imperfecti* respectively. Saccardo was supporting a tolerated use of the imperfect names beside the correct perfect name.

The matter, submitted to the International Botanical Congress of Vienna in 1905, was left to the International Commission of Botanical Nomenclature (Briquet 1905). The proposal of the Commission (Atkinson 1909) integrating the positions of Tulasne and Saccardo, was adopted at the Botanical Congress of Brussels in 1910, and became part of the Brussels International Code of Botanical Nomenclature (Briquet 1912) as Art. 49 bis. Art. 49 bis was the first rule of nomenclature for pleomorphic fungi. It recognized the predominance of the name of the perfect state of Ascomycetes and Basidiomycetes over the names of their imperfect states, and tolerated the use of the existing names of imperfect states, to be classified in "form-genera" and assessing them "only a temporary value". A later proposal made by the British mycologists (Anonymous 1929) to extend the rule 49 bis to the Zygomycetes and the Mastigomycetes (Phycomycetes), in order to make coherent the entire nomenclature of the fungi, was defeated at the Botanical Congress of Cambridge in 1930 (Briquet 1935, ICBN Art. 57).

The Stockholm Code (Lanjouw 1952), accepted for the first time, and ruled, in Art. 69, the creation of new names for the imperfect states of already named pleomorphic fungi, while the temporary value of such names was forgotten. The rule still evolved further to a very complex Art. 59 adopted in Edinburgh Code (Lanjouw 1966) and remained unchanged until 1981. The rule intended to separate the nomenclature of the imperfect fungi from that of the perfect fungi and to preserve the predominance and stability of the names of perfect species, by subordinating taxa of imperfect states of fungi as "form-taxa", and making invalid epithets transferred from form-species to perfect species and valid but illegitimate perfect names not typified by a perfect state in accordance with their protologue. In practice this version of Art. 59, was "undoubtedly one of the thorniest articles of the Code for mycologists to apply" (Korf 1982). It was "extraordinarily complicated, so that in some instances, up to three separate conclusions could be drawn regarding names to be used. There was a need for a drastic reconsideration of some basic principles" (Hawksworth 1984).
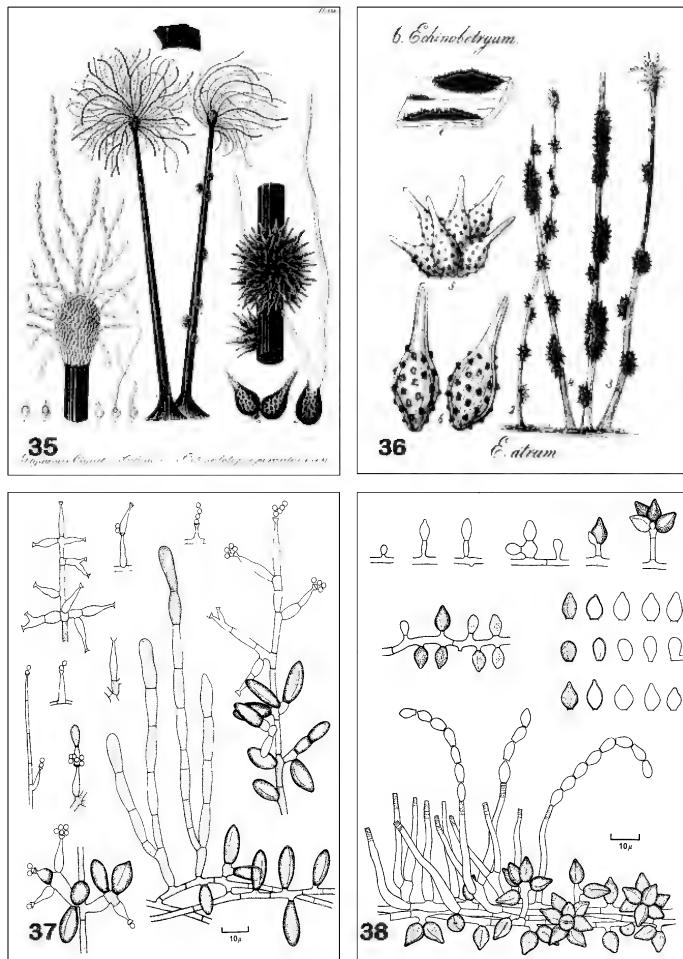
Fig. 35-38. Pleoanamorphy in fungi. 35. *Echinobotryum parasitans* Corda on *Stysanus caput-medusae* Corda after Corda , *Prachtflora* (1840); 36. *Echinobotryum atrum* Corda after Saccardo, *Fungi delineati* (1878); 37. *Wardomyces columbinus*, MUCL 6959, from culture; 38. *Mammaria echinobotryoides*, MUCL 7564, from culture.

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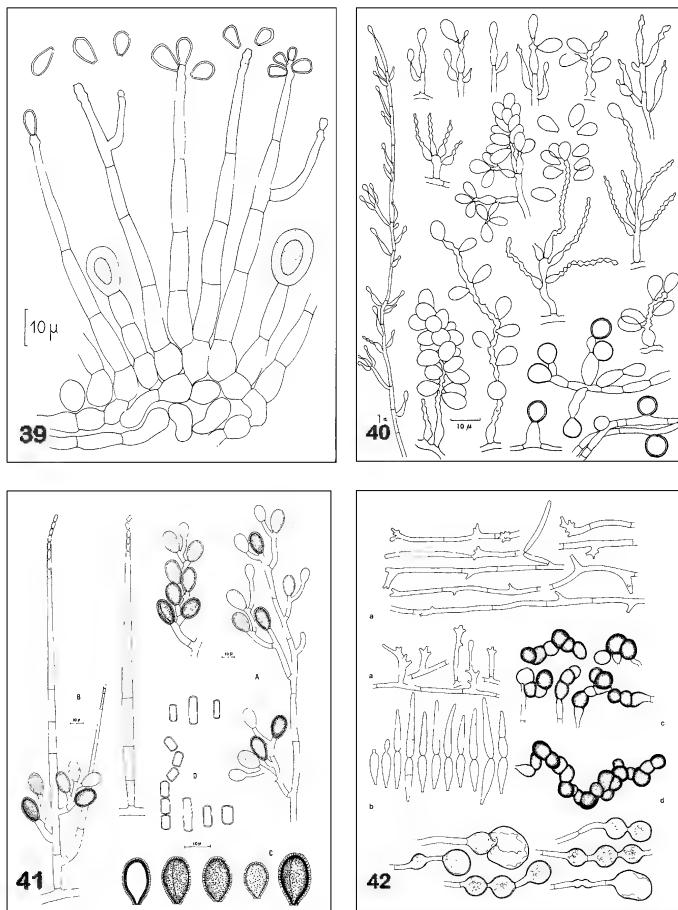
Fig. 39-42. Pleoanamorphic fungi. 39. *Raffaelea ambrosiae* Arx & Hennebert: 40. *Lomentospora prolificans* Hennebert; 41. *Chalaropsis punctulata* Hennebert; 42. *Isthmolongispora lanceata* De Hoog & Hennebert.

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3C. Revision of Art. 59 for the nomenclature of pleomorphic fungi

Such a reconsideration of basic principles of nomenclature I attempted to offer at the First Conference of Kananaskis, Canada, on the *Taxonomy of the Fungi Imperfecti* in 1969 (Kendrick 1971). In a paper on the *Pleomorphism in Fungi imperfecti,* I analysed the fundamentals of fungal nomenclature of pleomorphic fungi and 'pleoanamorphic' fungi (a new term at the time). The matter was not easy to put into words. Supported by Richard P. Korf at Cornell University, I formulated the concepts of "phase" versus "state", "form" of a fungus, of "whole fungus" versus "perfect state" and "imperfect states", of "botanical taxon" versus "form-taxon", and of "botanical nomenclature" versus "anatomical nomenclature", in order to define adequately the divergent options applied by fungal taxonomists in naming pleomorphic fungi, to deduce the nomenclatural implications of each option (Hennebert 1971).

A "sexual phase" and an "asexual phase" were distinguished in each fungus, even when the sexual phase is not actually known by man, both phases composing together the "whole fungus". The sexual phase is represented by a unique sexual form of reproduction, while the asexual phase is possibly represented by one or several asexual forms of reproduction or by nothing else than resting organs or mycelium.

The Edinburgh Code (Lanjouw 1966) treats the fungi according two distinct systems of nomenclature.

For the Mastigomycetes and Zygomycetes, the Code applies the "*botanical nomenclature*", meaning that only one name is given to "*the whole fungus*" as known, it being either sexual, or sexual and asexual, or only asexual, in strict application of the Linnean principle "one taxon - one name", based on what I call "*botanical typification*" governed by Art. 7: "*The nomenclatural type is not necessarily the most typical or representative element of a taxon*". An example is the genus *Mucor* that accommodates equally either sporangiosporic species, or zygo-sporangiosporic species ('imperfect' species), or even zygo-sporangio-blastosporic species, like *Mucor troglophilus* Zalar *et al.* (1997), no matter what part of the fungus the type specimen contains.

On the other hand, for the Ascomycetes, Basidiomycetes and Deuteromycetes, the Brussels (Briquet 1912) and subsequent Codes accept the use of several names for one particular fungus, one name for the sexual phase, typified by sexual reproduction and representing the "taxon", one or several names for the asexual phase typified by asexual reproduction and which represent one or several "form-taxa". The rule prescribes the predominance of the name of the sexual phase to designate the "taxon", excluding from consideration the names of "form-taxa". That system of nomenclature applies a different principle of typification than the rest of botanical nomenclature. Governed by Art. 59, the type of Ascomycete and

Basidiomycete names must be *obligatorily* the typical representative element of the taxon, i.e. the sexual fructification, while the type of Deuteromycete form-taxa is *restricted* to an asexual state, fructification or organ. In this system, names are clearly anatomical names, names of an anatomical part of the fungus. I called this system "*anatomical nomenclature*" with two distinct name statuses based on what I call here "*anatomical typification*". Even though the Code gives to names of sexual organs a botanical status, the resulting nomenclature of Ascomycetes or Basidiomycetes is not truly "botanical", for an obligation restricts typification, excluding from priority otherwise potentially competing names of asexual states.

Some fungal taxonomists refusing to create names for asexual states of pleomorphic fungi apply, to refer to them, an intermediate system called the "cross-reference naming system", using only the name of the form-genus associated to the sexual name (Hennebert 1971, 1983).

As a result of this analysis of the dual system of nomenclature, the First Kananaskis Conference, in 1969, decided the creation of a committee for reconsideration of Art. 59. This was formed as Subcommittee A of the International Mycological Association Nomenclature Committee at the First International Mycological Congress in Exeter, September 1971. The Subcommittee was composed of 12 members, with Dr Luella Weresub as Chairman.

3D. Anamorph, teleomorph, holomorph: new nomenclatural terms

In 1977, just playing with words in a conversation with Dr Luella Weresub in Ottawa, I proposed some less 'imperfect' terms than the anthropomorphic 'perfect' and 'imperfect', hopefully terms without value judgements and possibly objective and unequivocal to designate the anatomical parts of a fungus deserving a name. Urged on by Luella Weresub who believed in those terms, we published the terms "*anamorph*", "*teleomorph*" and "*holomorph*" to differentiate any fungal "*morph*" deserving a name (Hennebert and Weresub 1977). I was inspired by the term "anamorphosis" used by Donk (1960a, 1960b) following its first use by the Vienna International Commission of Botanical Nomenclature (Briquet 1905), and by the ICBNs of 1912, 1935 and 1947, for the asexual states of pleomorphic fungi. The term was preferred to the term "forma metagenetica" used by Saccardo (1889), and to the term "deuteroform" proposed by Shear (1929) as a replacement for the German "Nebenfrucht". The term "anamorph" was proposed for any asexual state of the "anamorphosis" (the asexual phase), the term "teleomorph" for the unique sexual state of the fungus and the term "holomorph" for the "whole fungus", including even all unknown "morphs". Any asexual fungus (imperfect fungus) is therefore an "anamorphic fungus", as lacking a teleomorph, either by a gap in our knowledge or having lost the capacity for sexual reproduction, irrespectively. The anamorphosis of a fungus can be

represented by one or several anamorphs that deserve names. When several, they were called "*synanamorphs*" by Hughes (1979). Fungi with synanamorphs are said "*pleoanamorphic*" and raise problems of nomenclature similar to those of pleomorphic fungi (Hennebert 1987c, 1991).

After several years of work, IMA Subcommittee elaborated a proposal for the modification of Art. 59 recommended by the Second International Mycological Congress in Tampa in 1977. In agreement with the proposal, the Nomenclature Session of the International Botanical Congress in Sydney in 1979 voted in favour of the use of the new terms *anamorph, teleomorph* and *holomorph*, in a new version of Art. 59. This, adopted by the Sydney Code (Stafleu 1981), simplified the rule, but reinforced the segregation of anamorphic nomenclature from teleomorphic (holomorphic) nomenclature of Ascomycetes and Basidiomycetes, based on their respective "anatomical typification". It clearly declared anamorphic names unpriorable in holomorphic nomenclature in case the anamorphic fungi were ever found to be sexual. It was the most conservative option, far from the true botanical nomenclature requiring the suppression of Art. 59, what I considered the final solution. New Art. 59 had however two advantages, to solve a serious nomenclatural knot by a clear practical line and, overall, to stimulate reconsideration of the basic principles of nomenclature of the fungi.

For plant pathologists, anamorphic names of plant pathogens often serve to designate plant diseases. Similarly, in medical mycology, names of mycoses commonly derive from the anamorphic name of the causal agent. Both practices are regrettable since they link disease names to a not infrequently changing reference basis. Using the practice as an argument in favour of an anatomical nomenclature and the maintenance of the Deuteromycetes is fallacious (De Vroey and Hennebert 1983). The true reason in maintaining Art. 59 was the fear of the major disturbance that its suppression and a return to unrestricted typification and absolute priority of names would cause in the present usual fungal nomenclature.

3E. Anamorph-teleomorph correspondence

Anxious to nurture a trend toward integration of conidial fungi into the taxonomy of Ascomycetes, Bellemère and Hennebert presented before the Mycological Congress in Tampa in 1977 a compilation of the various conidiogeneses known in the Discomycetes together with their connections with teleomorphic genera (Hennebert and Bellemère 1977, 1979).

In 1979, the Second Conference of Kananaskis considered "*The Whole Fungus*". Enlightened by the new terminology (Hennebert and Weresub 1979) and the new rules of nomenclature for pleomorphic fungi (Weresub 1979), a wider survey of anamorph-teleomorph connections in the Ascomycetes and Basidiomycetes was elaborated (Kendrick 1979). The survey showed the intricacy of the correspondences between anamorphic and teleomorphic genera, making the dreamed integration of the

Deuteromycetes into the Ascomycetes and Basidiomycetes an uneasy but challenging puzzle.

This is possibly due to lack of refined taxonomic criteria for the differentiation of genera at both sides. As far as anamorphs are concerned, a more detailed analysis of the *unitary parameters* of conidiogenesis, from the origin of the conidioma till the maturity of conidia, is needed. Hennebert and Sutton (1994) made such an attempt, which is still incomplete. A similar approach would presumably be helpful, too, in revision of teleomorphic genera.

A new survey of conidiogenesis in Ascomycete families by Sutton and Hennebert (1994) did not show any correspondence but, rather, heterogeneity between taxonomies, suggesting that reciprocal amendments might improve generic congruency. Using this approach, I obtained a strict correspondence between anamorphic and teleomorphic species of *Melampsora* (Hennebert 1964b). Also, through emphasis of characters discriminating new genera in the *Botrytis*-like complex, the anamorphic taxonomy could be appropriated to that of the correlated Discomycete teleomorphs (Hennebert 1973, Sutton and Hennebert 1994).

3F. Nomenclature of pleoanamorphic fungi

Pleoanamorphic fungi (synanamorphoses) raise the same problems as those of plemorphic fungi (teleomorphosis-anamorphosis). I studied a number of pleoanamorphic fungi, beside *Botrytis* species: *Ambrosiella xyleborii* and *Raffaelea ambrosiae* (Fig. 39) both with two kinds of conidia (von Arx and Hennebert 1965), *Chalaropsis punctulata* (Fig. 41) with sympodial thalloblastic conidia and *Chalara* phialidic thallic conidia (Hennebert 1967), *Spirosphaera* species with bulbiferous conidia disintegrating into secondary arthroconidia (Hennebert 1968a), *Echinobotryum atrum* (Fig. 35, 36), *Wardomyces columbinus* (Fig. 37) and *Mammaria echinobotryoides* (Fig. 38) with basipetally successive blastic conidia and phialidic or annelidic conidia (Hennebert 1968c), *Trichocladium* and *Thermomyces* species with thallobastic and philidic conidia (Hennebert 1971), *Lomentospora prolificans* (Fig. 40) with sympodial conidia and chamydospores (Fig. 35) (Hennebert and Desai 1974) and *Isthmolongispora lanceata* (Fig. 42) with sympodial conidia and its *Zalerion* thalloblastic morph (De Hoog and Hennebert 1983). They raised the still unanswered question of a monoanamorphic versus a pleoanamorphic definition of anamorphic genera.

Here also, I distinguished three systems applied by mycologists to pleoanamorphic fungi, from the botanical (pseudo-botanical) to the anatomical system with the intermediate cross-reference system (Hennebert 1971, 1987b). The new Art 59 of the Sydney Code (Stafleu 1981), which adopts the anatomical system for pleomorphic fungi, does not require the dissection of the anamorphosis into synanamorphs with distinct names, as long as the application of any anamorphic name corresponds to its type.

Therefore the choice of a monoanamorphic or pleoanamorphic definition of an anamorphic genus is free. It should be governed only by the perspective of a more natural classification (Weresub 1979). Examples exist in the botanical nomenclature of Entomophthorales where "one- state" and "three- state" species are disposed of and named in different genera in a unique taxonomy and nomenclature (Remaudière and Hennebert 1980).

To awaken consciousness to the nonsense of an anatomical system pushed so far as to dissect the anamorphoses of fungi and thus to mupltiply the names of synanamorphs, I took two extreme positions. Nathalie Buffin and myself decided naming provocatively the same fungus by two names, *Cylindrodendrum album* Bonorden and *Cylindrocarpon hydrophilum* Buffin and Hennebert, after it produced distinct conidiophores and conidia, although of the same phialidic conidiogenesis, under different culture conditions (Fig. 43) (Buffin and Hennebert 1984). The position actually conformed to anatomical nomenclature of anamorphic fungi. It is clear however that *Cylindrocarpon hydrophilum*, in a pseudo-botanical system, is a synonym of *Cylindrodendrum album*.

In non-conformity with that system, we described, under a unique name, *Basifimbria spinosa* Buffin and Hennebert, a fungus producing two kinds of conidia on the same conidiophore, and indicated, ironically, where eventually to cut the conidiophore with scissors to give the different parts different names(Fig. 44) (Buffin and Hennebert 1985, Hennebert 1987b, 1991).
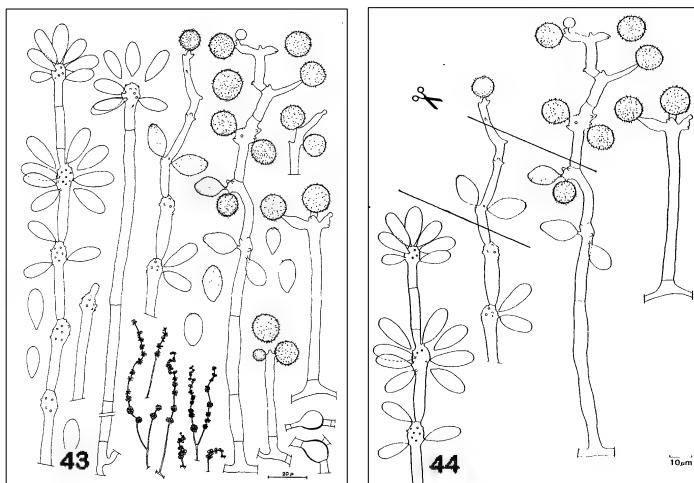
Fig. 43-44. *Basifimbria spinosa* Buffin & Hennebert. 43. Variability on the same culture medium.; 44. Variability or pleoanamorphy needing sectioning into pieces for three anamorphic names?

Obviously, good sense must prevail. The fundamental aim of naming living beings is indeed to make possible their recognition, inventory and classification, by giving each of them a single name and not a multiplicity of names (Hennebert 1991). I therefore prefer giving one name only to pleoanamorphic fungi. So I proceeded in naming and describing *Botrytis, Chalaropsis, Wardomyces, Mammaria, Humicola* species, using eventually a cross-reference generic denotation for what I considered the "secondary" conidiogenesis. Clearly the anatomical system of nomenclature, allowing a micro-dissection of the fungi, might "encourage les amateurs de gloriole, ceux qui se plaisent à voir leur nom imprimé" (De Candolle 1867). As asserted by mycologists 90 years ago, "names given to other states" than the teleomorph "have only a temporary value" (Briquet 1905), we should remember that all anamorphic names coined for pleomorphic fungi are by definition and typification of a subordinate status, some day in the future destined to become obsolete and to disappear (Hennebert 1987b).

3G. Towards a more natural classification

My dream remains to see the day when taxonomy and nomenclature of the Deuteromycotina (anamorphs and anamorphic fungi) shall be integrated with those of the Ascomycotina and Basidiomycotina (holomorphs) with the abandonment of names for anamorphs of teleomorphic fungi, in order to use only one name per fungus, the *nomen botanicum*. This dream I share with others. Already Weresub and Pirozynski (1979) declared "We trust that the time will come when our grasp of the morphology, biochemistry and genetics of all fungi will enable us to classify all anamorphic fungi botanically" with correlatively the demise of the Deuteromycota. After a critical analysis of the new Art 59 of the Sydney Code, I concluded: "The morphological diversity, the ontogenetic plasticity of forms and the unity of the chemotaxonomical characters expressed by the unique gene pool in the same fungus demonstrate clearly enough the artificial nature of anatomical, monoanamorphic taxa and the need for a more synthetic concept of the species to approach a more natural taxonomy" (Hennebert 1991).

When in August 1992, Reynolds and Taylor (1993) convened a conference at Newport, Oregon, on the theme of "*The Fungal Holomorph*", they had, as objective, the integration of Deuteromycotina into the taxonomy of Ascomycotina and Basidiomycotina. The trend was confirmed by a large majority of mycologists at the Newport Conference (Hawksworth 1993). The arguments brought in by the speakers were mostly biomolecular: the close phylogenetic relatedness between many anamorphic and teleomorphic fungi.

The push towards integration will stimulate interactive adjustment of both taxonomies and consequently the mixing of their nomenclature so as to reach a genus - form-genus, species - form-species correspondence. The

integration should mark different steps. The minimal step is to distribute the Deuteromycotina into two groups, the *Ascodeuteromycotina* and the *Basidiodeuteromycotina*, like *Cryptococcus* was segregated from *Candida*. A further step is to place those anamorphic genera near holomorphic genera in orders and families to which they belong: *Aspergillus* and *Penicillium* in the Eurotiales, Trichochomaceae, for instance. A next possible step would be to integrate biologically connected anamorph and teleomorph genera in unique genera, e.g., *Monilia* in *Monilinia*, *Botrytis* and *Amphobotrys* in *Botryotinia*; such genera should include holomorphic species and anamorphic species with their respective holomorphic or anamorphic names (Hennebert 1993).

In the integration process, what shall be the status of the anamorphic names? Two options can be conceived. One option might reject Art. 59, to anihilate anatomical typification in higher fungi; making any name of equal and concurrent status would realize a truly botanical nomenclature. Even were it applied from a future date, that option would create such a turn-over of fungal nomenclature, that it shall not be acceptable. The other option, the only feasible one, is to maintain the *anatomical typification* approved by Art. 59, which prescribes an obligate teleomorphic typification for holomorphic names of Ascomycotina and Basidiomycotina and a restricted anamorphic typification for anamorphic names of Asco- and Basidiodeuteromycotina, making the latter names forever non-synonymous and non-priorable, in other words "deprived of nomenclatural status by typification" in regard to holomorphic names. In such an integrated classification, the names of anamorphs of pleomorphic fungi will disappear by use of the holomorphic names. The names of those anamorphic fungi still lacking any connection to teleomorphs ("anamorphic holomorphs", according Reynolds 1993) should remain treated as "pseudo-botanical" names (Hennebert 1991) for so long as a related teleomorph remains undiscovered.

At the Newport Conference in August 1992 , the terms *anamorph*, and *teleomorph* were disputed by Sutton (1993) and Hawksworth (1993), who preferred the term "mitosporic" and "meiosporic" to qualify the fungus or parts of the ascomycete or basidiomycete fungus, the term "mitospore" replacing the 150 year-old term "conidium". After the matter was raised again at the Conference on *Ascomycete Systematics* in Paris in 1993, Korf and Hennebert (1993) answered the argument and emphasized the disastrous situation that such deviations in terminology already foreseen by Weresub and Hennebert (1979) would create. The terms anamorph and teleomorph are *not* karyological but *nomenclatural* terms, which adequately serve the nomenclature of higher fungi. Fungal spores are said meiotic or mitotic depending on their direct *parental* nuclear division, the meiotic spores being those spores containing one or more of the four meiotic nuclei. The adjectives "meiosporic" cannot correctly qualify Ascomycotina and Basidiomycotina, as spores of most Ascomycetes and numerous Basidiomycetes are mitotic, containing none of the four nuclei of meiosis. In prospect of the conceivable and hopefully predictable integration of the

Deuteromycotina in the Ascomycotina and the Basidiomycotina, and as long as the rule of anatomical typification is maintained in these fungi, the terms anamorph and teleomorph will remain necessary.

CONCLUSION
Taxonomy and nomenclature of the fungi, reasons for a Mycotheque

Fungal taxonomy and fungal nomenclature are linked intimately. Both will really progress towards a natural classification of the fungi if they are based on an accurate and extensive knowledge of the fungi based on their morphology and development. This requires repeated collections of fungi in the field and their isolation in pure culture on various media and under diverse conditions, and on their morphological and morphogenetic description in pure culture in such media and under such conditions, using a harmonized and unequivocal terminology, and finally the application of a correct name in regard to the taxonomic literature and the rules of nomenclature.

For such achievement, a mycotheque is the basic and necessary tool for mycology. It includes a herbarium, culture collection, iconotheque, library, data bases and expert systems. Surely, fungal systematics will not be successfully completed without collaborative morphological, biological and genomic studies. But, at the least, it should first be started with field work and morphological descriptions. Genomic analysis is only an additional, currently and unfortunately luxurious, approach.

The task for fungal taxonomists is *urgent:*, for the undiscovered biodiversity of the fungi will not wait. Only 5 % of the mycoflora consisting of a presumed 1,500,000 species is known. Much of that important and still undiscovered diversity (more than 1,400,000 species) lies in tropical zones, mainly in tropical forests (Hawksworth 1991, 1994). The actual destruction rate of the tropical forests is such that that reservoir of biodiversity shall be entirely burnt up within 100 years, by 2100 AD (Hennebert 1996a). At the current rate of 14 new species published per day by the existing mycological community, about 350 years of work by such a community will be needed if the objectives remain what they are today. Spending manpower, time and money in elaborating a genetic phylogeny, a pretended natural classification of the fungi from a non-representative sample (5%) of the fungal world is clearly an impossible vision. The goal is not to achieve the impossible, but rather it is in the field we must spend our energies. Let's go to work. Wonders are waiting for us before being all too soon incinerated.

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INDEX OF FUNGAL NAMES

Species names in summaries, tables, plates and dendrograms are not indexed.
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